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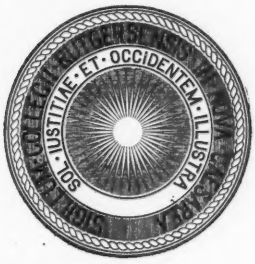

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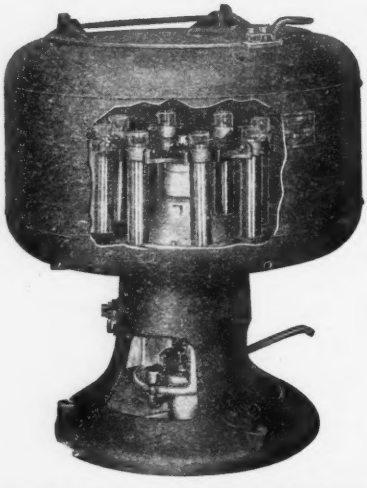
For Mechanical Analysis of Soils



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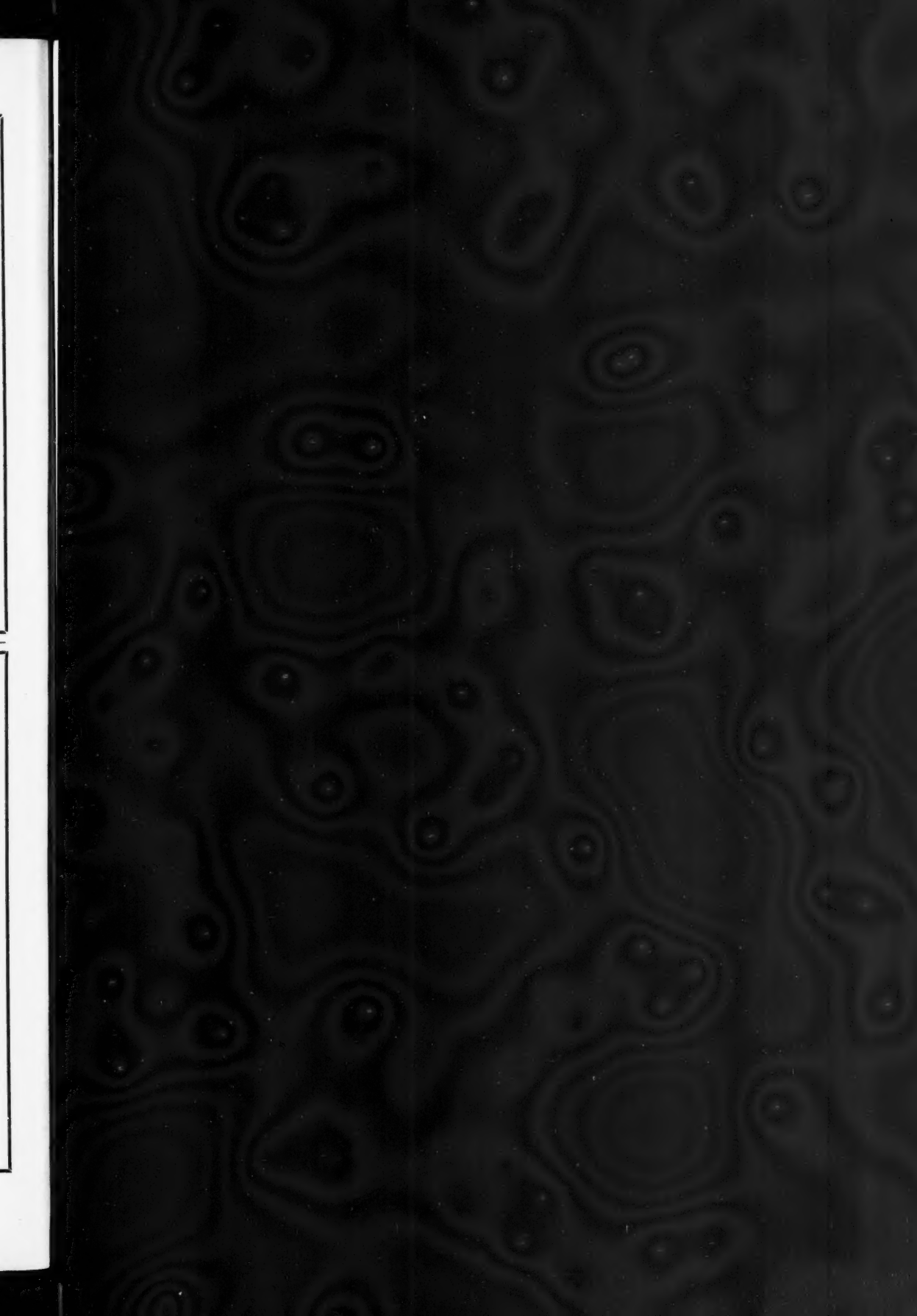

FACTORS INFLUENCING THE DETERMINATION OF SULFATES IN SOIL

C. T. HIRST AND J. E. GREAVES

Utah Agricultural Experiment Station

Received for publication June 15, 1921

Many methods have been proposed for the determination of sulfates. Some are ideal for the purpose for which they were originated, but if they are applied to other substances or used under other conditions they do not give accurate results. Some of the methods are cumbersome and complex and consume considerable time. The methods may be divided into two classes, namely, the gravimetric and the volumetric. The gravimetric method depends on the low solubility of barium sulfate. The dissolved sulfate is treated with an excess of a soluble barium salt, the chloride, the nitrate, or the hydroxide—although the chloride is usually used—and the precipitated barium sulfate is filtered, washed, ignited, and weighed. It has also been suggested to precipitate sulfate as strontium sulfate but the high solubility of the resulting salt renders the method of little value.

Fresenius (11) states that the

> . . . . exact estimation of sulfuric acid as barium sulfate is by no means so easy and simple as it was formerly supposed to be, but requires great care and attention. This is due to three causes: First, the barium sulfate is found to be far more soluble than was believed in solutions of free acids and of many salts; second, it is extremely likely to carry down with it foreign salts which are themselves soluble in water; third, when the precipitate has once separated in an impure state it is often very difficult to purify it completely.

The solution must contain only a little free hydrochloric acid and no nitric acid.

Kolthoff and Vogelenzang (26) claim that the solubility of barium sulfate is increased by a rise in temperature, by the presence of nitric acid, and to a lesser extent by hydrochloric acid, and that dry ignition in platinum causes large losses. Kato and Noda (24) obtained inexact results in the presence of equivalent solutions of sodium chloride, potassium chloride, and ammonium chloride, the potassium chloride giving the largest error. The error increased with the addition of alkali until the alkali chloride was present in the ratio of 2 mols to 1 of the sulfate. Sacher (40) found the barium sulfate much more soluble in potassium choride solution than in water, but his results do not confirm the interference of the chloride. De Sornay (9) found the usual method of determining sulfates to be inexact, particularly if much iron or aluminum were present. Krieble and Mangium (25) obtained concordant

results in the determination of sulfates in potassium sulfate in the presence of strong hydrochloric acid, although the results are slightly low. In the presence of sodium chloride and 2 cc. of concentrated hydrochloric acid added to 10 cc. of the potassium sulfate solution the results were high, but with a larger concentration of hydrochloric acid more nearly concordant results were found. Blumenthal and Guernsey (5) found that various strengths of acid did not influence the results to any great extent but that low acidity was preferable. Karaoglanow (21) found low results in the presence of nitric acid and large amounts of hydrochloric acid, while high results were obtained in the presence of potassium salts and ferric chloride.

The same worker (22) found that in dilute solution the precipitation of sulfuric acid as barium sulfate depends on the concentration of acid present and on the excess of barium chloride, the barium sulfate decreasing with the increase of barium chloride. The decrease is not due to the presence of barium chloride since barium sulfate is practically insoluble in it. High results were obtained in the presence of relatively large amounts of nitric acid and hydrochloric acid. Potassium chloride caused low results, but potassium nitrate high results. Potassium sulfate gave low results, but when hydrochloric acid was present the results were too high. The presence of potassium chloride and potassium sulfate together gave low results whether or not hydrochloric acid was present. The presence of sodium, ammonium, aluminum, and magnesium does not affect the results. Ferric chloride gives results much too low. However, in very dilute solutions containing hydrochloric acid the effect of iron is small.

Creighton (8) found aluminum to increase the weight of barium sulfate. De Sornay (9) found the determination of sulfate as barium sulfate inexact in the presence of iron and aluminum. The precipitation of sulfates as barium sulfate in the presence of iron salts yields a double sulfate of iron and barium which on ignition decomposes with a loss of sulfur trioxide (27, p. 275). Williams (47) obtained higher results in determining the sulfates in soil solutions when the iron and aluminum were removed than when they were left in. On account of their solvent action on barium sulfate the precipitate also is likely to become contaminated by the iron salts present.

The causes of these varying results have been variously explained. Ruppin (37) attributes the high results obtained to the occlusion of barium chloride and the low ones obtained under other conditions to the solubility of barium sulfate in the foreign salts present. Kolthoff and Vogelenzang (26) claim that the occlusion of barium chloride, the nitrates, calcium, iron, and potassium salts is chemical in nature and cannot be washed out after ignition as claimed by some. The error in the presence of phosphates is due to formation of barium phosphate. Kato and Noda (24) think the error caused by potassium is due to the fact that potassium sulfate and barium sulfate both crystallize in the same system and thus form mixed crystals. They favor the adsorption theory since the formula obtained for the ratio of salt to acid, and the percent-

age difference between the results obtained in the presence and in the absence of potassium chloride is very similar to the adsorption formula, it is therefore probable that the adsorption takes place during the precipitation of the barium sulfate. No such adsorption occurs in the presence of magnesium chloride.

According to Allen and Johnston (1) the solubility of barium sulfate is not greatly influenced by alkali salts, but the acidity is a more important factor. It is their opinion that adsorption is an important factor since barium chloride and "free" sulfuric acid are frequently identified as impurities in barium sulfate precipitates. Nitrates also are likely to be adsorbed, producing high results.

Blumenthal and Guernsey (5) found that there was little difference in the use of 5- and 10-per cent barium chloride added to solutions of constant volume and acidity, but the rate of adding it was somewhat more important. Phelps (33) claims that by precipitating in a hot neutral solution with barium chloride the contamination of the barium sulfate precipitate with foreign ions may be almost avoided and that the precipitate contaminated by ions such as potassium, sodium, and ammonium may be converted into barium sulfate by treatment with sulfuric acid, evaporation, and extraction of the alkali sulfate with water. Karaoglanow (23) obtained the best results when the barium chloride was added during a period of not less than 1.5 minutes, while Blumenthal and Guernsey (5) obtained the best results when the barium chloride was added at the rate of 5 cc. per minute. Allen and Johnston (1) recommend that the barium chloride be added to the hot sulfate solution at such a rate that 2 gm. of barium sulfate is precipitated in 3 to 6 minutes.

The error accruing in the determination of sulfates as barium sulfate may at times amount to 5 per cent of the whole (27, p. 277) and because of the time element involved in making many analyses, numerous volumetric methodshave been suggested. These in general, however, lack the accuracy of the gravimetric method (27, p. 277). Mohr (29) proposed to determine sulfuric acid by adding an excess of standard barium chloride and titrating the excess of barium chloride by means of standard sodium carbonate using phenolphthalein as indicator. This method is inaccurate (42, p. 311) in the presence of metals other than the alkalies or acid ions such as phosphates, carbonates, and oxalates which form insoluble salts with barium. Consequently it would be useless for the determination of sulfates in alkali soils.

Clemm (7) modified Mohr's method in that he precipitated the excess of barium chloride with standard sodium carbonate, filtered off the precipitated barium sulfate and carbonate, then titrated the excess of sodium carbonate with standard acid and from that calculated the sulfates present. This modification is open to the same objections as the original method. Bohlig (4) proposed a similar method in which he precipitated sulfate with barium carbonate, thereby forming an equivalent quantity of alkali carbonate which, after filtration, was estimated by means of standard acid. Here the presence of oxalates, arsenates, chromates, and phosphates is objectionable. Gross-

man (13) precipitated the sulfates with barium hydroxide, removed the excess of barium with carbon dioxide, thereby leaving an amount of alkali carbonate equivalent to the sulfates present, which was titrated with standard acid. This method is open to the same objections as Mohr's method. Augur and Gabillon (3) proposed to reduce the sulfuric acid and sulfates with hydriodic acid and to estimate the hydrogen sulfide liberated with standard iodine.

Rivett (35) precipitated sulfates as barium sulfate by the use of moist barium oxalate and estimated the amount of soluble oxalates set free with standard permanganate solution. Obviously, if there are metals present which form insoluble oxalates or acid ions which form insoluble barium compounds the results will be inaccurate.

The sulfates were determined by Marboutin and Moulinie (28) by precipitation with an excess of barium chloride, the excess being precipitated with potassium chromate and the excess potassium chromate titrated either with iodine or arsenious acid. An almost identical method was proposed by Telle (43) who used barium chloride, potassium dichromate, potassium iodide, and sodium thiosulfate solutions, titrating the liberated iodine with sodium thiosulfate. This latter method is very much like one proposed earlier by Hinman (15) and by Andrews (2), who precipitated the sulfates with a hydrochloric acid solution of barium chromate. The excess of barium chromate was removed by adding either ammonia or calcium carbonate to the boiling solution. The solution was then filtered and the alkali chromate present equivalent to the sulfuric acid, reduced with potassium iodide, the iodine liberated being titrated with standard thiosulfate. The barium chromate must be free from soluble chromates and must contain no soluble barium salt (44, p. 717) or barium carbonate.

Holliger (17) used the same method only varying the procedure slightly. Roemer (36) modified the method in that he titrated the alkali chromate resulting from the precipitation of the sulfate with ferrous ammonium sulfate using potassium ferric cyanide as an indicator. Komarowsky (20) applied the method, with modified manipulation, to the analysis of waters with good results. Ruys (38) applied it to the determination of alkali sulfates. North (32) modified the method in that he used standard solutions of barium chloride and potassium dichromate. He found it to be usable in the presence of the following ions: Si, K, Na, NH_4, Cr, Mg, Ca, Sr, Zn, Cd, Hg, Al, Ni, Co, Cl, PO_4, and B_4O_7. Sborgi and Sotgia (4) varied the procedure in that they used a standard solution of barium chloride and an unstandardized solution of potassium dichromate. Then by running two series and making a calculation they arrived at the sulfates present.

Wildenstein (46) and Repiton (34) precipitated the sulfate with standard barium chloride. The excess of barium is precipitated by standard potassium chromate, the potassium chromate itself forming the indicator. In applying this method (11, p. 438) to solutions containing the sulfates of magnesium, zinc, or cadmium, the sulfates are first dissolved in ammonia with the addition

of ammonium chloride heated with a little calcium chloride to remove any carbonate present, then the barium chloride is added, and finally the potassium chromate.

Edmunds (10) precipitated the sulfates with an excess of decinormal barium nitrate. The excess of barium is precipitated with potassium chromate and the excess of the latter then precipitated with an excess of decinormal silver nitrate. The excess of silver is determined in a portion of the filtrate.

Vaubel (45), followed by Müller (30), Müller and Durkes (31), and later by a large number of other workers, proposed to estimate sulfuric acid by means of benzidine hydrochloride. The sulfate is precipitated as benzidine sulfate which is filtered off, after which the acid set free is titrated in the filtrate by standard alkali.

According to Treadwell and Hall (44, p. 715) in this method there are two sources of error: first, the benzidine sulfate is not insoluble in water so that there is considerable sulfuric acid not precipitated, and second the precipitate tends to adsorb some benzidine hydrochloride. They influence the results of the analysis in opposite directions and neither wholly compensates for the other.

EXPERIMENTAL

A consideration of the various volumetric methods indicated that the chromate method was the most likely to give satisfactory results for the following reasons: First, it is easy of manipulation; second, the reagents are common and are not especially expensive; third, the end-point is sharp. The method is fairly rapid. A comparison has been made, therefore, of the barium sulfate gravimetric method and the chromate volumetric method on varying concentrations of sulfuric acid in pure solution. The effect of various salts likely to occur in natural soils also was considered. First a comparison was made of the volumetric and gravimetric methods in which the same quantity of sulfate was determined in solutions of varying concentrations. These results are given in table 1. Each result is the average of six closely agreeing determinations. In the actual work the sulfuric acid was determined in 10 cc. of each concentration. The results in the $1N$, $0.5N$, and $0.1N$ solutions have therefore been multiplied by 2, 4, and 20, respectively. Any error, therefore, made in the analysis is multiplied by these factors. The barium sulfate here, as elsewhere mentioned in this paper, was precipitated by adding the required amount of hot barium chloride drop by drop to the hot sulfate solution without stirring. The samples were digested for about two hours, usually left to stand over night, and filtered the next morning. The precipitates were washed with hot water until free of chloride as indicated by silver nitrate. The sulfates in the volumetric method were precipitated by adding a hydrochloric acid suspension of barium chromate until a drop of ammonium hydroxide indicated an excess of barium chromate. The solutions were kept near the boiling point for half an hour after which the excess of barium chromate was precipitated while

the solution was hot with concentrated ammonium hydroxide. The solution was cooled, made up to a definite volume without filtering and an aliquot part taken for analysis. No correction was made for the solubility of barium chromate since it is small and in the presence of the large excess of chromate ion would be very much smaller. The solubility of barium chromate in mol per liter at room temperature is 1.5×10–5 so that the solubility product is 2.3×10–10. If we have, say, 0.1*M* alkali chromate present which is 85 per cent ionized, the concentration of the barium ion would be only $2.7 \times 10-9$ mol per liter, and the concentration of the chromate ion coming from the barium chromate would be the same. This quantity may properly be neglected.

It is true that a small error would be introduced on account of the volume occupied by the precipitate, but this procedure was followed to avoid the washing of the precipitated barium sulfate and chromate as well as to avoid making the correction for the solubility of barium chromate under varying conditions, such as volume of filtrate and temperature.

TABLE 1

Sulfuric acid recovered from varying strengths of sulfuric acid by the barium sulfate gravimetric and the barium chromate volumetric methods

VOLUME OF H_2SO_4	APPROXIMATE STRENGTH	NUMBER OF ANALYSES MADE	H_2SO_4 RECOVERED	
			Gravimetric method	Volumetric method
cc.	*N*		*gm.*	*gm.*
10	2.0	6	1.0110	0.9899
20	1.0	6	1.0076	1.0038
40	0.5	6	1.0160	0.9258
200	0.1	6	1.0260	0.9110

If the sulfuric acid recovered by the barium sulfate method be taken as 100 per cent in each case, then the chromate method when applied to a 2*N* solution, yielded 97.9 per cent of the sulfuric acid present. From a normal solution 99.6 per cent was obtained, where as from the half normal and decinormal solutions respectively, 91.1 and 88.8 per cent of the acid was recovered. It seems, therefore, that the chromate method is best suited to solutions of about normal concentration. Acid of this concentration was used in this work.

THE EFFECT OF CHLORIDES

It is often desirable to determine sulfates in soil, water, and other material containing varying quantities of soluble salts. It is essential to know the influence of these salts upon the accuracy of the two methods.

Accordingly, varying quantities of approximately 2*N* solutions of the chlorides of sodium, potassium, aluminum, and ferric iron were added to 10 cc. solutions approximately normal sulfuric acid. The results are recorded in table 2. In each case these results are the average of three closely agreeing determinations.

TABLE 2

Sulfuric acid recovered in the presence of varying amounts and kinds of chlorides by the barium sulfate gravimetric and barium chromate volumetric methods*

STRENGTH OF CHLORIDE SOLUTION ADDED TO 10 CC. OF APPROXIMATELY NORMAL H_2SO_4	SULPHURIC ACID RECOVERED							
	Sodium chloride		Potassium chloride		Aluminum chloride		Ferric chloride	
	Gravimetric	Volumetric	Gravimetric	Volumetric	Gravimetric	Volumetric	Gravimetric	Volumetric
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
12.5 cc. 2*N* sol. + 2.5 cc. H_2O	0.5015	0.5048	0.4982	0.4940	0.5068	0.4805	0.5032	0.4174
10.0 cc. 2*N* sol. + 5.0 cc. H_2O	0.5012	0.5041	0.4979	0.4987	0.5076	0.4834	0.5034	0.4589
7.5 cc. 2*N* sol. + 7.5 cc. H_2O	0.5010	0.5017	0.4978	0.4987	0.5046	0.4834	0.5024	0.4612
5.0 cc. 2*N* sol. + 10.0 cc. H_2O	0.5004	0.5017	0.4994	0.4953	0.5028	0.4918	0.5019	0.4612
2.5 cc. 2*N* sol. + 12.5 cc. H_2O	0.5015	0.4983	0.5004	0.4950	0.5028	0.4994	0.5020	0.4781
Average	0.5011	0.5021	0.4987	0.4963	0.5048	0.4877	0.5026	0.4550

* Sulfuric acid recovered from 10 cc. of untreated H_2SO_4: 0.5038 gm. by barium sulfate gravimetric method; 0.5019 gm. by barium chromate volumetric method.

Sodium chloride has but little effect. In most cases this effect was slightly depressing. The average of the five sets of determinations by the gravimetric method compared with that obtained with the pure solution, shows 0.5011 gm. of sulfuric acid as against 0.5038 gm. found in the latter case. We may assume, then, that sodium chloride in the concentrations used has a slightly depressing effect on the determination of sulfuric acid by the gravimetric method. The volumetric method when used on the same concentrations gave in most cases slightly high results, but the average of the five sets of results, 0.5021 gm. agrees almost exactly with that obtained on the pure solution.

Potassium in the form of chloride, in every concentration and in both methods, exerted a slightly greater depression than did sodium chloride. The averages show 0.5011 gm. of sulfuric acid in the presence of sodium chloride against 0.4987 gm. in the presence of potassium chloride by the gravimetric method, and 0.5021 gm. of sulfuric acid in the presence of sodium chloride against 0.4963 gm. in the presence of potassium chloride by the volumetric method.

Aluminum chloride exerted an opposite effect with the gravimetric method. This positive error is most pronounced in the higher concentrations and decreases to about normal in the lower ones. In the average there is a small positive departure from that obtained in pure solution. By the volumetric method, however, results were obtained which are just the reverse. All the values but one, 0.4994, are lower than any obtained in the presence of either sodium or potassium salts or pure solutions by either method. The discrepancy is most marked in the higher concentrations and as the amount of aluminum chloride decreases, the error grows smaller until in the lower concentrations almost the normal value is obtained.

The effect of ferric chloride was similar to that of aluminum chloride. So far as can be judged by the data obtained, these two ions have about the same effect on the barium sulfate method, but the iron causes much lower results with the volumetric method than the aluminum does.

The causes of these abnormal results have been investigated by many workers, among whom are Allen and Johnston (1) and Johnston and Adams (19). They find, in opposition to the work of some investigators, that whereas the solubility of barium sulfate is but slightly influenced by the presence of alkali chloride, the amount of alkali sulfate occluded by the barium sulfate particles is increased. This conclusion is drawn because there is present in the precipitate before ignition (*a*) chloride almost certainly barium chloride, (*b*) sodium sulfate and (*c*) sodium hydrogen sulfate. The amount of these substances they find present depends on first, the composition of the original solution, second, the method of precipitation and third the length of time between precipitation and filtration. A high acidity will tend to reduce the amount of alkali sulfate occluded with the precipitated barium sulfate but will have a greater solvent action. If the barium sulfate is precipitated rapidly and cold, the particles will be small, thus producing a maximum surface on

which the occlusion may take place. If a considerable time elapses between precipitation and filtration, the equilibrium between the solid and dissolved phases will result in recrystallization and a consequent smaller amount of alkali sulfate occluded.

Hulett and Duschak (18) think the abnormal values follow from the formation of complex salts, but Blumenthal and Guernsey (5) do not believe they are explained either by the complex salt or that normal and bisulfates are occluded.

THE EFFECT OF NITRATES

The effect of the nitrates of the same elements is much more marked both by the gravimetric and the volumetric methods. These results are recorded in table 3. In each case the same amount of sulfuric acid is used. The results are the average of three to six determinations. In some cases the duplicate samples did not agree closely. This, according to Allen and Johnston is due to the occlusion of nitrates the selective adsorption of which points to a solid solution.

Sodium nitrate increased the amount of sulfuric acid found. This increase became smaller as the amount of sodium nitrate decreased, but even in the lowest concentration used it was considerable. The abnormal value is more marked in the volumetric than in the gravimetric method, and this is to be anticipated since in the hydrochloric acid solution in which the iodine is titrated there would be liberated nitric acid which would serve to oxidize the iodide ion to free iodine. Potassium nitrate caused a much more pronounced error than did sodium nitrate. On the average, this influence was more marked with the gravimetric method. To obtain concordant results a close adherence to set conditions must be maintained, and even under the best conditions the results will be high. Aluminum nitrate also was found to exert a pronounced influence; it gave too high results with the gravimetric method, but iu most cases caused low results with the volumetric method. With ferric nitrates, the gravimetric results are very slightly lowered while volumetric results are very markedly depressed. Here, as with the other nitrates studied, the greatest errors either positive or negative occurred in the higher concentrations, the values approaching normal in the lower ones.

When the sulfates are precipitated as barium sulfate in the presence of iron, aluminum or chromium salts, the sulfates of these metals are occluded and on ignition decompose, liberating sulfur trioxide and produce low results. The magnitude of the error depends on the concentration of the original solution and on the rate at which the precipitation takes place, the occlusion being greater in the presence of nitrates than in the presence of chloride. The nitrates, however, not only greatly further the occlusion of sulfates but are themselves occluded. This causes large positive errors. According to Hulett and Duschak (18) positive errors are caused by the formation of complex salts containing nitrogen.

TABLE 3

Sulfuric acid recovered in the presence of varying amounts and kinds of nitrates by the barium sulfate gravimetric and the barium chromate volumetric methods*

STRENGTH OF NITRATE SOLUTION ADDED TO 10 CC. OF APPROXIMATELY NORMAL H_2SO_4	SULPHURIC ACID RECOVERED							
	Sodium nitrate		Potassium nitrate		Aluminum nitrate		Ferric nitrate	
	Gravimetric	Volumetric	Gravimetric	Volumetric	Gravimetric	Volumetric	Gravimetric	Volumetric
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
12.5 cc. 2*N* sol. + 2.5 cc. H_2O	0.5165	0.5213	0.5655	0.5634	0.5186	0.4852	0.5010	0.4550
10.0 cc. 2*N* sol. + 5.0 cc. H_2O	0.5143	0.5196	0.5560	0.5442	0.5182	0.4909	0.5028	0.4786
7.5 cc. 2*N* sol. + 7.5 cc. H_2O	0.5078	0.5172	0.5413	0.5408	0.5108	0.4983	0.5040	0.4915
5.0 cc. 2*N* sol. + 10.0 cc. H_2O	0.5113	0.5156	0.5286	0.5304	0.5111	0.5037		0.5027
2.5 cc. 2*N* sol. + 12.5 cc. H_2O	0.5096	0.5136	0.5220	0.5068	0.5034	0.5135		0.4960
Average	0.5119	0.5175	0.5427	0.5371	0.5124	0.4983	0.5026	0.4848

* Sulfuric acid recovered from 10 cc. of untreated H_2SO_4: 0.5038 gm. by barium sulfate gravimetric method; 0.5019 gm. by barium chromate volumetric method.

The ignited barium sulfate precipitated in the presence of ferric nitrate was invariably colored by the ferric oxide produced. An attempt was made to remove this colored material by washing and decantation with dilute hydrochloric acid. The results as shown in table 4 are the average of three determinations. Precipitation was made in the evening and filtration took place the next forenoon. The weight of the precipitate decreased but was still somewhat high in the presence of aluminum, and in the case of the iron they were still colored by the iron oxide although the results are low compared with the untreated sulfuric acid. No doubt part of the loss was due to the solvent action of hydrochloric acid on the barium sulfate.

Higher results than those given in table 4 were obtained when barium sulfate precipitated in the presence of iron salts was digested in dilute hydrochloric acid for 2 hours and filtered, as compared with those left to digest over night and filtered the following morning. In this case the barium sulfate recovered

TABLE 4

Effect of Hydrochloric acid digestion on precipitated barium sulphate

STRENGTH OF NITRATE SOLUTION ADDED TO 10 CC. APPROXIMATELY NORMAL H_2SO_4	BARIUM SULFATE RECOVERED			
	Aluminum	Nitrate	Ferric nitrate	
	Original weight	Weight after digestion with HCl.	Original weight	Weight after digestion with HCl
	gm.	*gm.*	*gm.*	*gm.*
12.5 cc. 2*N* nitrate + 2.5 cc. H_2O	0.5086	0.5052	0.5042	0.4984
10.0 cc. 2*N* nitrate + 5.0 cc. H_2O	0.5084	0.5059	0.5046	0.4994
7.5 cc. 2*N* nitrate + 7.5 cc. H_2O	0.5078	0.5048	0.5042	0.4993
5.0 cc. 2*N* nitrate + 10.0 cc. H_2O	0.5084	0.5056		
2.5 cc. 2*N* nitrate + 12.5 cc. H_2O	0.5050	0.4997		

varied from 0.5225 gm. in the presence of 12.5 cc. of 2*N* solution of ferric chloride (total solution 50 cc.) to 0.5172 when 5 cc. of 2*N* ferric chloride was added. In the results reported in table 4 the greater time between precipitation and filtration has favored the more complete reaction between the occluded sulfates and the precipitating reagent. No doubt the recrystallization of the of the barium sulfate liberated part of the occluded materials. This positive effect of iron was observed also when the same quantity of ferric chloride was added to sulfuric acid of varying strengths.

Thus when 10 cc. of 2*N* ferric chloride was added to 10 cc. of approximately normal solution of sulfuric acid, 0.5168 gm. of sulfuric acid was obtained as against 0.5056 in the absence of iron. When approximately 2*N* sulfuric acid was substituted for the normal solution, 1.0185 and 1.0110 gm. respectively of sulfuric acid was recovered. In each case the total volume of solution was 50 cc.

More nearly correct results may be obtained by precipitating the iron and aluminum salts by the use of ammonium hydroxide and the sulfates as usual

with hot barium chloride. After the precipitation is complete the hydroxides are dissolved with 10 cc. of concentrated hydrochloric acid and the usual procedure followed. The results are recorded in table 5. Each chloride result is the average of three samples and each nitrate result the average from five to eight determinations.

By this procedure somewhat low results were obtained in the presence of ferric chloride, the average being 0.4911 gm. as against 0.4922 gm. in the pure solution. The concordance of samples, however was good as there was a maximum variation of 4 mgm. of barium sulfate between the highest and lowest in any set of three, and in no other set did it exceed 1.5 mgm.

When ferric nitrate was present, the average of eight determinations of sodium sulfate recovered was almost exactly the same as that obtained on the pure solution but there was a much larger variation between results than when

TABLE 5

Sodium sulfate recovered from 10 cc. of a solution of sodium sulfate treated with varying amounts of ferric and aluminum chlorides and nitrates*

SOLUTION			SODIUM SULFATE RECOVERED			
0.69 *N* Na_2SO_4	2 *N* salt	H_2O	Ferric chloride	Ferric nitrate	Aluminum chloride	Aluminum nitrate
cc.	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
10	12.5	2.5	0.4914	0.4920	0.4937	0.4992
10	10.0	5.0	0.4912	0.4911	0.4934	0.4965
10	7.5	7.5	0.4918	0.4925	0.4931	0.4957
10	5.0	10.0	0.4916	0.4927	0.4917	0.4938
10	2.5	12.5	0.4906	0.4920	0.4932	0.4936
Average.....			0.4911	0.4921	0.4930	0.4958

* The average of fifteen determinations showed that 10 cc. of the pure solution contained 0.4922 gm. of sodium sulfate.

the chloride was used In some cases the difference between the highest and lowest was as much as 13 mgm.

With aluminum chloride present, higher results were obtained than in the presence of ferric chloride. The concordance of samples however, was even better, the maximum difference being 3 mgm. of barium sulfate in any one set and in most cases less than 1 mgm. The average weight obtained, 0.4930 gm., was only slightly greater than that obtained from the pure solution and easily within experimental error.

When we turn to the study of aluminum nitrate we again observe that the nitrates caused higher results than chlorides. Here the average weight of sodium sulfate obtained in the presence of aluminum nitrate was 3.6 mgm. higher than that obtained on the pure solution. The concordance among samples was poor, the maximum difference was 7 mgm. in the presence of the maximum quantity of aluminum nitrate. It seems, however, that by this procedure more nearly accurate results are obtained.

THE EFFECT OF THE PASTEUR-CHAMBERLAND FILTER

The ultimate object of this work is the determination of sulfates in soil, particularly as applied to alkali soils. The first essential for such study is the preparation of a clear filtrate. In our work on the nitrates (12) and chlorides (16) clarifying agents, such as alum and lime, were used with excellent results. For obvious reasons, however, neither of these substances could be used for clarifying solutions in which sulfates were to be determined, particularly if there were any appreciable amounts of sulfate present as is the case with many alkali soils. It seemed then, that the only procedure was to clarify the solution with the Pasteur-Chamberland filter.

Before using the filters, it was desirable to know whether or not they adsorb the sulfates. Solutions of sodium sulfate, potassium sulfate, and magnesium sulfate were made up. Portions of these solutions were then filtered through new, unused filters which had previously been soaked in distilled water and

TABLE 6

Potassium sulfate and magnesium sulfate recovered from filtrates of potassium sulfate and magnesium sulfate from Pasteur-Chamberland filters

SALT USED	FIRST 10 cc.	SECOND 10 cc.	THIRD 10 cc.	FOURTH 10 cc.	FIFTH 10 cc.	SIXTH 10 cc.	10 cc. OF FILTRATE ABOVE 60 cc.	UNFILTERED PORTION
cc.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
K_2SO_4	0.3549	0.3519	0.3517	0.3519	0.3519	0.3520	0.3532	0.3523
$MgSO_4$	0.2921	0.2908	0.2902	0.2904	0.2911	0.2904	0.2889	0.2889
Na_2SO_4							0.4927	0.4931

then dried. The remaining portions were left unfiltered. Five filters were used, the sulfates being determined gravimetrically in the unfiltered and in successive six 10– cc. portions of the filtrate and also in that portion which came through the filter above 60 cc.

The first 10 cc. of the filtrate contains more potassium sulfate than the unfiltered part, this no doubt is due to the adsorptive action of the filter, for there is but 0.0004 gm. difference in 10 cc. in favor of the filtered samples. The average of the seven filtered samples is 0.35253 gm. of potassium sulfate in 10 cc. as against 0.3523 gm. in 10 cc. of the unfiltered, a difference of but 0.23 mgm. in 10 cc. of solution.

In the work with magnesium sulfate the first 60 cc. filtered gave a more concentrated solution. That portion above 60 cc. is identical with the unfiltered portion, namely, 0.2889 gm. of magnesium sulfate per 10 cc. of the solution. In the potassium sulfate solution the concentration occurred only in the first portion but the first six magnesium sulfate portions are all more concentrated than either the latter part of the filtrate or the unfiltered portion.

THE SOIL

The soil used was a brown loam obtained from Benson Ward, Utah. There was but little sulfate present; consequently, sodium and magnesium sulfate were added and the sulfate determined by leaching 25 gm. of the soil. After mixing the sulfates into the soil the mixture stood 3 days before analyzing. Portions of it were agitated with water in a mechanical shaker and later filtered through Pasteur-Chamberland filters, after which the sulfates were determined by the barium sulfate gravimetric and the chromate volumetric methods. These results are given in table 7 and are reported as percentages of sulfuric acid based on the air-dried soil.

TABLE 7

Sulfuric acid recovered from soil

SOIL TREATMENT	SULFURIC ACID RECOVERED		
	Sulfate gravimetric method	Chromate volumetric method	Ratio of sulfuric acid recovered by the volumetric chromate method as compared with the barium sulfate gravimetric method
	per cent	*per cent*	(*vol.*) : (*grav.*)
Leaching	2.234	2.017	94.32 : 100
Agitation and filtration through Pasteur-Chamberland filter	2.266	2.187	96.52 : 100

RATIO OF SOIL TO WATER

The smallest ratio of soil to water which is necessary to extract the maximum quantity of sulfates from the soil was determined by preparing mixtures in which the ratio of soil to water was 1:5, 1:10, 1:15, and 1:20, respectively.

These mixtures were agitated in a mechanical shaker for 1 hour, then filtered through Pasteur-Chamberland filters. The sulfates were determined by both the chromate volumetric and the barium sulfate gravimetric methods. The results are recorded in table 8 which shows the percentages based on the air-dried soil of sulfates calculated to sulfuric acid recovered.

So far as this soil is concerned, containing as it does a mixture of about equal parts of sodium and magnesium sulfates, as great a quantity of sulfates is extracted from a mixture of the ratio of 1 part of soil to 5 parts of water as from mixtures of higher ratios. It is true that with the ratio of 1:15 the amount is somewhat higher, but this, it seems, must be due to experimental error since in the ratio 1:20 it falls back again to almost exactly the value it had in the 1:5 ratio. These slight variations may be due to unequal distribution of the sulfate in the soil, although the sulfates were finely pulverized and well mixed by spading them into the soil which was subsequently subjected to prolonged grinding and mixing in a ball mill.

TABLE 8

Sulfates calculated as sulfuric acid recovered from mixtures of soil and water in various ratios

RATIO	SULFURIC ACID RECOVERED		
	Gravimetric barium sulfate method	Chromate volumetric method	Ratio of sulfuric acid recovered by the volumetric chromate method as compared with the barium sulfate gravimetric method
	per cent	*per cent*	(*vol.*) : (*grav.*)
1– 5	2.310	2.280	98.70 : 100
1–10	2.292	2.195	95.08 : 100
1–15	2.346	2.292	97.70 : 100
1–20	2.319	2.288	98.66 : 100

TIME OF SHAKING

Another question arose. How long must soil be agitated in order to obtain the maximum amount of sulfates possible with the ratio of soil to water used? In order to obtain some concrete data concerning the ordinary alkali soils, fifteen samples of the soil used in previous sections were made up in the ratio of 1 part of soil to 5 parts of water. Three of these samples were shaken 10 minutes, three 20 minutes, three 30 minutes, three 40 minutes, and three 50 minutes. The results are expressed in table 9.

TABLE 9

Percentages of sulfates, based on the air-dry soil and calculated as sulfuric acid, extracted by 5 parts of water to 1 part of soil and shaken for various lengths of time

TIME SHAKEN	BARIUM SULFATE GRAVIMETRIC METHOD	CHROMATE VOLUMETRIC METHOD	RATIO OF SULFURIC ACID RECOVERED BY THE VOLUMETRIC CHROMATE METHOD AS COMPARED WITH THE BARIUM SULFATE GRAVIMETRIC METHOD
min.	*per cent*	*per cent*	(*vol.*) : (*grav.*)
10	1.987	1.398	70.36 : 100
20	2.267	2.237	98.68 : 100
30	2.195	2.133	97.18 : 100
40	2.313	2.240	96.80 : 100
50	2.288	2.137	93.40 : 100

In our study of the nitrates (12) and chlorides (16) we found that nothing was to be gained by agitating the soil for more than 10 minutes but here equilibrium is not reached in that time, the maximum quantity being obtained after 40 minutes of agitation. This result was obtained from a solution not nearly saturated with the salt used. For example, at 0°, 100 gm. of water will dissolve 26.9 gm. of anhydrous magnesium sulfate, or 76.9 gm. of the heptahydrate, or 4.8 and 55.59 gm. of the corresponding salts of sodium. Under the present conditions there is present but 2.25 per cent of sulfates (about half sodium sulfate and half magnesium sulfate) calculated as sulfuric acid. Consequently, the solution does not approach saturation and yet the results show

that at least 40 minutes is necessary to reach equilibrium. If, as frequently happens, soils are rich in gypsum, then much longer shaking will be necessary to attain equilibrium in any ratio of soil to water that we wish to use.

It has been pointed out in the literature (27) that, in general, the volumetric methods for the determination of sulfates lack the accuracy of the barium sulfate gravimetric method. We found this to be true. Except in the presence of nitrates where nitric acid is liberated which oxidized part of the iodide present, consequently giving high results, the chromate volumetric method gives lower results than does the barium sulfate gravimetric method. The amount of this negative variation depends on the concentration of the solution. This may be observed by referring again to table 1. If the sulfuric acid obtained by the gravimetric barium sulfate method in each concentration is taken as 100 the volumetric chromate method is found to recover 97.92, 99.62, 91.13, and 88.80 per cent, respectively, from the 2*N*, 1*N*, 0.5*N* and 0.1*N* solutions of sulfuric acid.

The most nearly concordant results are obtained with solutions of about normal concentration. Here almost as much sulfuric acid is recovered by the chromate volumetric as by the barium sulfate gravimetric method. This ratio falls off as the concentration becomes either greater or smaller, the smallest percentage occurring in the 0.1*N* solution where only about 89 per cent of the sulfuric acid is recovered. It seems, therefore, that the volumetric chromate method is best suited to solutions of about normal concentrations. When the methods are applied to soil solutions more nearly concordant results under varying concentrations are obtained. This may be observed by referring to table 8 column 4. The sulfates recovered, calculated as sulfuric acid as determined by the volumetric method, vary from 98.7 per cent in the ratio 1:5 to 95.08 per cent in the ratio 1:10. But this decrease cannot be due to the difference in concentration since in the ratio 1:20, 98.66 per cent as much by this method as by the barium sulfate gravimetric method is recovered. This same variation is observed in the "determination of sulfates in the soil," by leaching and by agitation and filtration in which we obtained 94.32 and 96.52 per cent, respectively. In this leaching experiment it is probable that more organic matter is dissolved. In a chromic acid solution this would be oxidized and gives somewhat lower results.

In table 9 which gives data on the time of shaking, the recovery is shown to vary from 93.4 to 98.7 per cent. The volumetric chromate method gives as an average of all the results obtained on soil solutions 96.7 per cent as much sulfates calculated as sulfuric acid as does the gravimetric-barium sulfate method on the same solutions.

Since this method is considerably more rapid than the gravimetric method it seems that it might be used where a large number of sulfate determinations are to be made and a correction applied, depending upon the type of soil and the quantity of sulfates present.

SUMMARY

1. It requires a longer time of agitation to reach equilibrium in soil solution in which sulfates are to be determined than is necessary with chlorides (16) or nitrates (12). The time will depend on the kind and quantity of sulfates present.

2. The solution may be clarified by the use of either the filter or centrifuge. Lime or alum may not be used.

3. In the present case a ratio of 1 part of soil to 5 parts of water was all that was necessary for satisfactory extraction but this may vary greatly with different types of soil.

4. The chromate volumetric method recovers smaller quantities of sulfates than are obtained by the gravimetric method.

5. Since the chromate volumetric method is fairly rapid and easy of manipulation it may well be used for the determination of sulfates in soil solutions. Where the chromate volumetric method is used and a fair degree of accuracy is desired a factor must be used to correct for presence of aluminum, iron and nitrates.

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THE INFLUENCE OF MOISTURE AND SOLUBLE SALTS ON THE BACTERIAL ACTIVITIES OF THE SOIL

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In a previous article (1) we have shown that, in twenty-two soils varying widely in physical composition, maximum ammonification took place when the soil contained 60 per cent of its water-holding capacity as defined by Hilgard; nitrification was at a maximum when the soil contained between 50 and 60 per cent. Many of the soils showed two maxima for nitrogen fixation, viz., one, 50 to 60 per cent of total water-holding capacity and the other, 70 to 80 per cent. It is the purpose of this paper to consider the influence of various soluble salts upon the water requirements of the ammonifying and nitrifying organisms.

SOIL

The soil used in this work, taken from the college farm, is of a sedimentary nature. It was deposited by streams laden with debris from the near-by mountains which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil, given in table 1, showed that it was a sandy loam very high in acid-soluble constitutents, but not excessively high in water-soluble constituents. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium and there was a fairly large quantity of iron present. In fact, all the elements of plant-food were present in abundance, except nitrogen which occurred in only small quantities. The soil was very productive and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The nitrogen-fixing powers of the soil were above the average and previous work had shown it to have an intensely interesting bacterial flora.

The water-holding capacity of the soil, as determined by the Briggs' modification of the Hilgard (3) method, was 45 per cent. From 10 to 45 cc. of sterile water was added to the air-dried soil. This gave relatively the percentages of moisture stated in terms of water-holding capacity of the soil, that are listed in table 2 for the various determinations. However, there must have been a slight variation from set to set, depending upon the dryness of the soil used. This would shift the point of optimum either higher or lower as the case may be. Each entire set, however, was maintained at the same moisture content. Hence, they were directly comparable and varia-

tions within a set were due to the salts added and not to experimental errors. The various moisture contents which were used in the study are given in table 2.

TABLE 1

Physical and chemical composition of soil

PHYSICAL COMPOSITION		CHEMICAL COMPOSITION	
Constituent	Per cent	Constituent	Per cent
Coarse sand, above 1 mm.....	17.69	Insoluble matter.....	66.69
Fine sand, 1.0–0.03 mm.....	37.39	Potash (K_2O).....	0.55
Coarse silt, 0.03–0.01 mm.....	15.19	Soda (Na_2O).....	0.49
Medium silt, 0.01–0.003 mm.....	10.36	Lime (CaO).....	7.41
Fine silt, 0.003–0.001 mm.....	10.32	Magnesia (MgO).....	4.15
Clay below, 0.001 mm.....		Ferric oxide (Fe_2O_3).....	2.93
Moisture and loss.....	9.05	Alumina (Al_2O_3).....	3.49
		Phosphorus Pentoxide (P_2O_5).....	0.25
		Sulfur trioxide (SO_3).....	0.07
		Carbon dioxide (CO_2).....	7.62
		Humus.....	2.18
		Total nitrogen.....	0.15

TABLE 2

Approximate moisture contents at which ammonification and nitrification were tested

AMMONIFICATION		NITRIFICATION	
Moisture added	Moisture content in terms of moisture-holding capacity	Moisture added	Moisture content in terms of moisture-holding capacity.
per cent	*per cent*	*per cent*	*per cent*
45	100	35	77
40	88	30	66
35	77	25	55
30	66	20	44
25	55	15	33
20	44	10	22
15	33		
10	22		

METHOD OF EXPERIMENTATION

In order to determine the ammonifying power of the soil, 100-gm. portions of the soil, 2 gm. of dried blood, and quantities of various salts, as indicated in the several graphs, were weighed into sterile tumblers which were kept covered with petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to the desired 10 to 45 per cent in 5 per cent increments. The samples were incubated at 28°–30°C. for days and the ammonia determined by transferring the mixture with 400 cc. of distilled water to Kjedahl flasks adding 2 gm. of magnesium oxide and distilling into 0.1 *N* sulfuric acid.

The nitrifying power of the soils was determined in similar mixtures which were incubated for 21 days. The moisture content varied from 10 to 35 per cent and was restored every 7 days to the initial percentages. The nitric nitrogen was determined as follows (2):

> The contents of the beaker, together with 500 cc. of distilled water and 2 gm. of alum, were placed in quart Mason jars and agitated for five minutes in a shaker.
>
> An aliquot part (100 cc.) of the supernatant liquid was pipetted off, and, together with 2 cc. of a saturated solution of sodium hydroxide, was evaporated to about one-fourth of its original volume to free it from ammonia. To this were added 50 cc. of ammonia-free water, 5 gm. of "iron-by-hydrogen," and 30 cc. of sulfuric acid (sp. gr. 1.35). The neck of the reduction flask was fitted with a 2-hole stopper through which passed a 50-cc. separatory funnel and a bent tube which dipped into a vessel containing water in order to prevent mechanical loss. The acid was slowly added and allowed to stand until the rapid evolution of hydrogen was over. It was then heated to boiling for ten minutes. The contents of the side vessel were returned to the reduction flask before the reaction was complete, thus insuring the complete reduction of any nitrates which may have been carried over with the first violent evolution of the hydrogen. The contents of the reduction flask were transferred to Kjeldahl flasks, neutralized with sodium hydroxide, and distilled into standard acid. The excess of acid was titrated back with standard alkali, lacmoid being used as an indicator. Controls were run on all the reagents, including the alum used as a flocculent.

AMMONIFICATION

The results obtained in soils treated with the chloride, carbonate, sulfate, and nitrate of sodium are given in figure 1, as the averages of six or more closely agreeing determinations. Ammonification reached its maximum in soil containing 30 per cent of moisture in the case of chloride and carbonate, but in the case of the sulfate and nitrate maximum ammonification was reached at a moisture content 5 per cent higher. The relative toxicity of the various salts decreased as the percentage of water in the soil increased and was most pronounced in the soil treated with carbonate and least in that treated with nitrate. In the presence of sodium chloride and with 10 per cent of moisture there was only 67 per cent ammonia produced as compared with the untreated, whereas with 45 per cent of moisture there was 103 per cent ammonia produced as compared with the untreated soil. The relations between the other salt-treated soils and the check, considering the untreated as 100 per cent, were as follows:

Sodium carbonate	10 per cent of moisture,	53 per cent of ammonia
	45 per cent of moisture,	116 per cent of ammonia
Sodium sulfate	10 per cent of moisture,	53 per cent of ammonia
	45 per cent of moisture,	93 per cent of ammonia
Sodium nitrate	10 per cent of moisture,	32 per cent of ammonia
	45 per cent of moisture,	50 per cent of ammonia

This is what one would expect where the toxicity is due mainly to osmotic pressure, as is the case with these salts.

The results obtained for the chlorides, carbonates, sulfates, and nitrates of potassium are given in figure 2. The untreated soil, together with that receiving potassium nitrate and potassium carbonate, reached their maximum ammonifying efficiencies at a moisture content of 30 per cent, whereas the soils treated with sulfate, chloride, or nitrate reach their maximum at 35 per cent.

The relative toxicity of the different salts varied widely with the moisture content of the soil. Soil treated with potassium sulfate bore a nearly constant

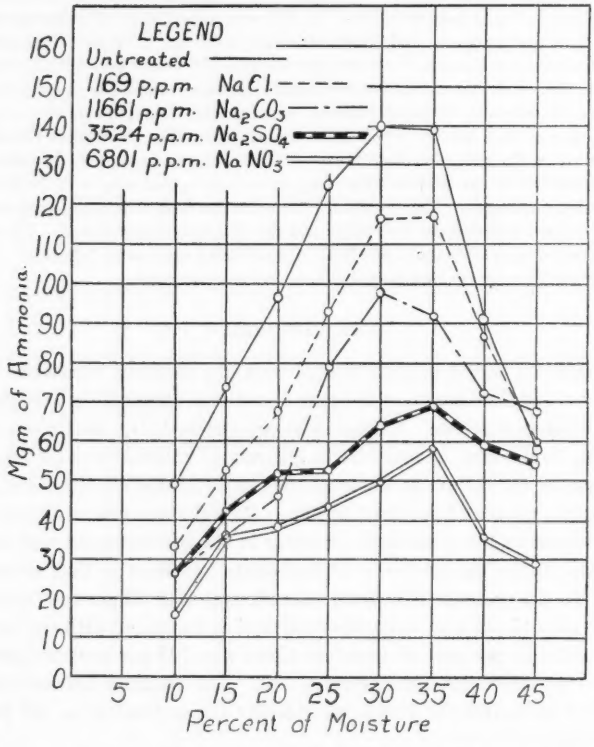

FIG. 1. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT SODIUM SALTS, AND VARYING PERCENTAGES OF WATER

relationship throughout to the untreated soil. The chloride and carbonate in soil with 10 per cent moisture decreased ammonification nearly 50 per cent, but with 45 per cent moisture the results became 91.8 and 142.0 per cent, respectively. In other words a sufficient quantity of potassium chloride in a soil with 10 per cent of moisture reduces ammonification about 50 per cent, but when the moisture content was raised to 35 per cent it had only a very slight retarding influence. With the carbonate the results were even more far-

reaching, for a quantity which, in a soil with low moisture content reduced ammonification 50 per cent, actually increased the relative quantity of ammonia produced when the moisture became excessive. These facts point strongly to the conclusion that in the case of potassium chloride and potassium carbonate the toxicity was due to osmotic effect, whereas in the case of potassium nitrate some physiological action must have occurred which was not offset by the mere dilution of the salt.

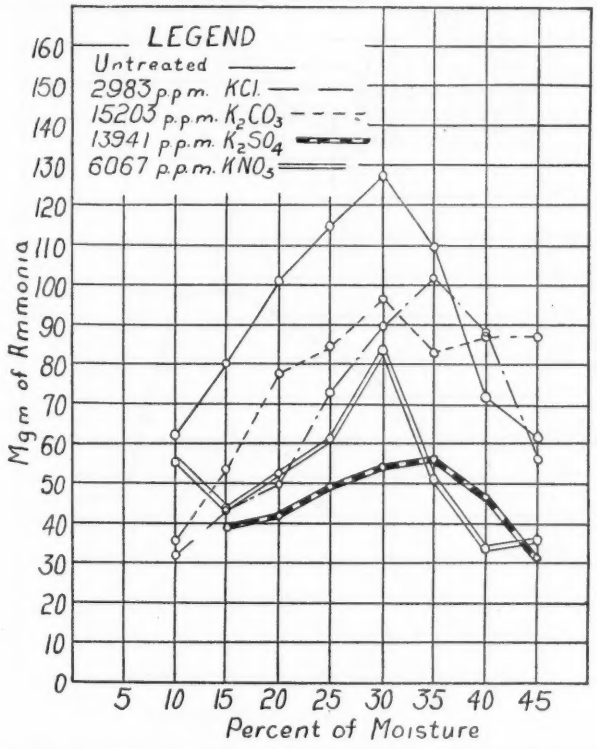

FIG. 2. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT POTASSIUM SALTS, AND VARYING PERCENTAGES OF WATER

The addition of chloride, carbonate, sulfate, or nitrate of calcium to a soil did not change the water requirements of that soil for optimum ammonification. This is shown in figure 3. The untreated and treated soils were all at their maximum ammonifying power at a moisture content of 30 per cent. There was no perceptible relative decrease in toxicity of these salts with increase of water-content. This is what one would expect if the toxicity is due to a change in the calcium-magnesium ratio. The same condition obtained

in the magnesium series (fig. 4). Both series bore out the conclusion that the calcium-magnesium ratio was an important factor. The nature of the cation, however, was an important factor, for the nitrate became relatively more toxic with increasing dilution both in the calcium and magnesium series. It has been quite evident throughout the consideration of all series that the nitrates were obeying a different law than were the chlorides, carbonates, and sulfates, and while the toxicity of the nitrate was due mainly to a chemical

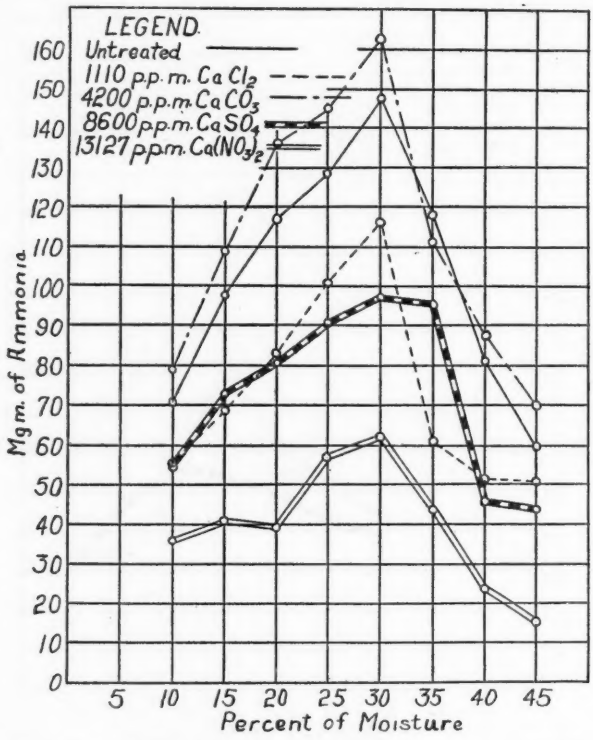

FIG. 3. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT CALCIUM SALTS, AND VARYING PERCENTAGES OF WATER

or physiological effect upon the protoplasm, the action of the chlorides, sulfates, and carbonates was due more largely to an osmotic effect. Magnesium carbonate becomes relatively less toxic with dilution, whereas the calcium carbonate becomes relatively more toxic. This is probably due to there being a preponderance of calcium in the soil, and the narrowing of the calcium-magnesium ratio makes it more nearly ideal for ammonification. The magnesium-sulfate-treated soil appears to require less moisture for optimum activity than do the soils receiving other salts.

So far in the discussion we have been comparing the action of compounds having the same electro-positive but various electro-negative ions. Hence, the results considered have given us an insight into the influence of the anions chloride, sulfate, nitrate, and carbonate upon the ammonifying efficiency of the soil. Soil samples were, therefore, treated with compounds in which the anion was a constant and the cation a variable. Figure 5 shows that results of treatment with chlorides. The addition of sodium chloride to the soil

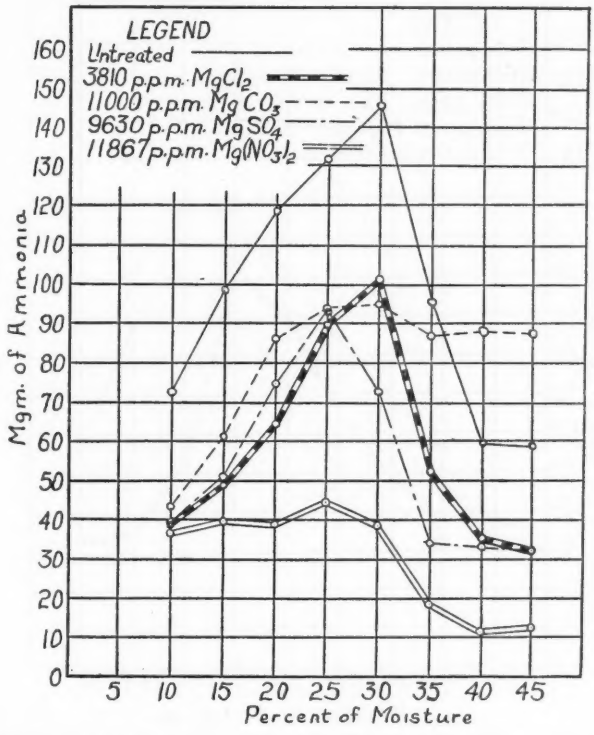

FIG. 4. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT MAGNESIUM SALTS AND VARYING PERCENTAGES OF WATER

shifted the point of maximum ammonification from 30 to 35 per cent content of moisture. In all the other soils, the point of maximum ammonification was at a moisture content of 30 per cent. The ratio of ammonia produced in the untreated soil to that produced in the salt-treated soil became narrower in the case of sodium and potassium chloride as the addition of water increased, whereas this ratio became greater with magnesium and calcium chloride. This increase of toxicity was very pronounced with calcium and indicated

that the calcium and magnesium ion added to this soil was a true poison to ammonifying bacteria. Potassium and sodium, however, acted more through the increasing of osmotic pressure in the soil and their relative effect was offset by the increasing of the water content.

The point of maximum ammonification with sodium nitrate, as with sodium, chloride, was raised 5 per cent, as shown in figure 6. Soil treated with magnesium nitrate reached its maximum ammonification when the water content

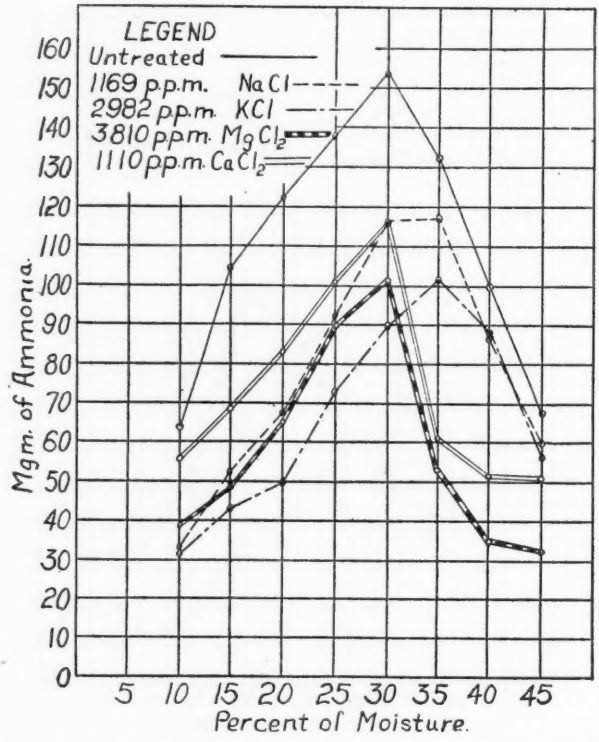

FIG. 5. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT CHLORIDES, AND VARYING PERCENTAGES OF WATER

of the soil was 25 per cent. The untreated soil, together with that receiving potassium and magnesium nitrate, reached its maximum at 25 per cent of water. As the quantity of water applied to the soil was increased, the ratio of ammonia produced in the untreated soil to that produced in the salt-treated soil increased in every case except that of sodium nitrate. Potassium nitrate-treated soil containing 10 per cent of water produced 86 per cent as much ammonia as the untreated soil, whereas with 45 per cent of water it produces

50 per cent; magnesium nitrate with 10 per cent of water produced 57 per cent as much ammonia and with 45 per cent of water, 22 per cent; calcium nitrate with 10 per cent of water produced 55 per cent as much ammonia and with 45 per cent of water, 30 per cent of ammonia. On the other hand, the sodium nitrate-treated soil receiving 10 per cent of water produced 24 per cent as much ammonia as the untreated soil and with 45 per cent of water, 50 per cent. In other words, the addition of water to a soil containing potassium, mag-

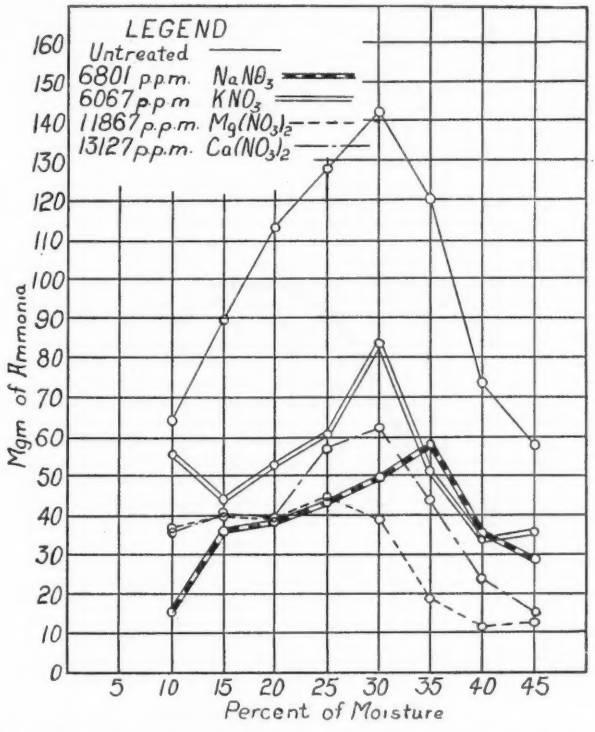

FIG. 6. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT NITRATES, AND VARYING PERCENTAGES OF WATER

nesium, or calcium nitrate increased the quantity of ammonia produced up to a certain maximum which lies between the moisture contents of 25 and 30 per cent. It decreased, however, the relative quantity of ammonia produced as compared with the untreated soil. This fact bears out the proposition that the toxic action of the nitrates was due in a large measure to a physiological effect upon the living protoplasm of the microörganisms of the soil and not to physical changes produced in the soil.

The results obtained with the sodium, potassium, magnesium, and calcium carbonate-treated soils as compared with the untreated soil are given in figure 7. In each soil its maximum ammonification was reached when the soil contained 30 per cent of water. However, the quantity of ammonia found in the different soils varies widely and at every concentration of water the calcium chloride-treated soil produced more ammonia than the untreated soil. The sodium carbonate-treated soil with a 10 per cent moisture content

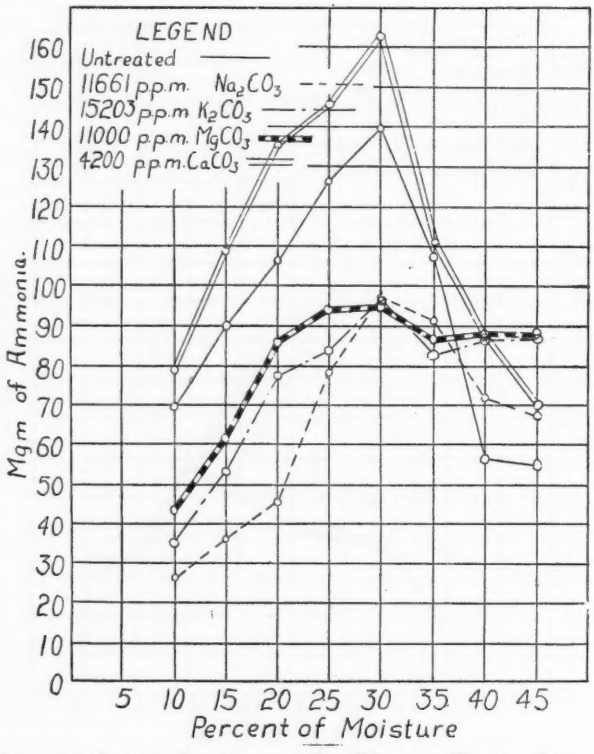

FIG. 7. CURVES OF AMMONIA PRODUCTION IN SOIL WITH AND WITHOUT CARBONATES, AND VARYING PERCENTAGES OF WATER

produced only 37.6 per cent as much ammonia as the untreated soil with a similar water content, whereas with 45 per cent of water the former produced 122.4 per cent as much as the latter. This indicated that the mere dilution of the carbonate decreased toxicity. The phenomenon appeared throughout the carbonate-treated soil, which is just the opposite to that occurring in the soils treated with chlorides and nitrates. This increase was real and not merely apparent, as would be the case if the nitrifying powers of the soil had decreased proportionately with the increased water content.

No such uniform effect was observed in the case of soil receiving sulfates, as is brought out in figure 8. The untreated soil and that receiving calcium sulfate reach their maximum ammonifying activity with a 30 per cent water content, whereas soil receiving sodium or potassium sulfate were not at their maximum efficiency until their moisture contents were raised to 35 per cent. This would seem to indicate that alkali soils containing sodium or potassium sulfate would be more productive with high than with low water content.

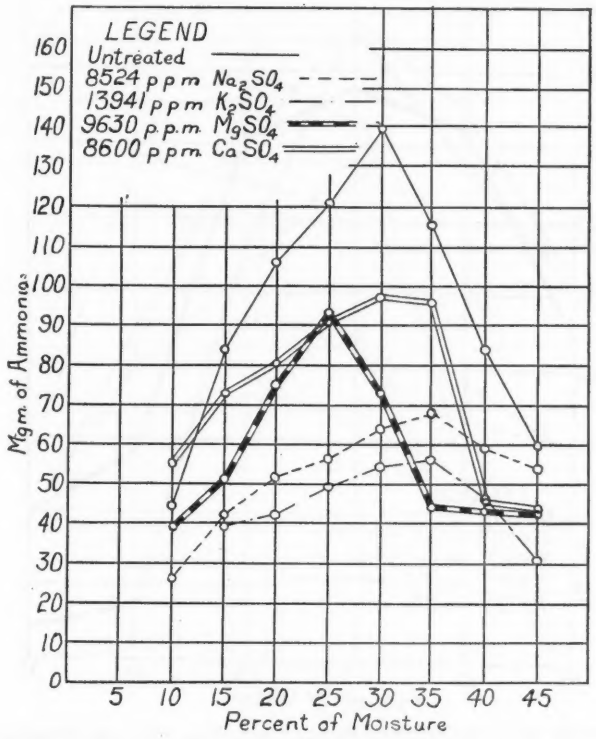

FIG. 8. CURVES OF AMMONIA PRODUCTION IN SOIL, WITH AND WITHOUT SULFATES, AND VARYING PERCENTAGES OF WATER

Soils containing magnesium sulfate were more productive of ammonia when the water content was only 25 per cent. The ratio of the amount of ammonia produced in the salt-treated soil to that produced in the untreated soil increased slightly with increased moisture content in soil treated with potassium sulfate and to a greater extent in soil treated with sodium sulfate. However, the ratio decreased in soil to which magnesium or calcium sulfate was added.

NITRIFICATION

The same soil with the same combinations of salts was tested for nitrifying powers. The results obtained with soil receiving chloride, carbonate, sulfate, and nitrate of sodium and with untreated soil is given in figure 9. On the ordinate is given the number of milligrams of nitric nitrogen produced in 100 grams of soil, whereas on the abscissa is given the percentage of water

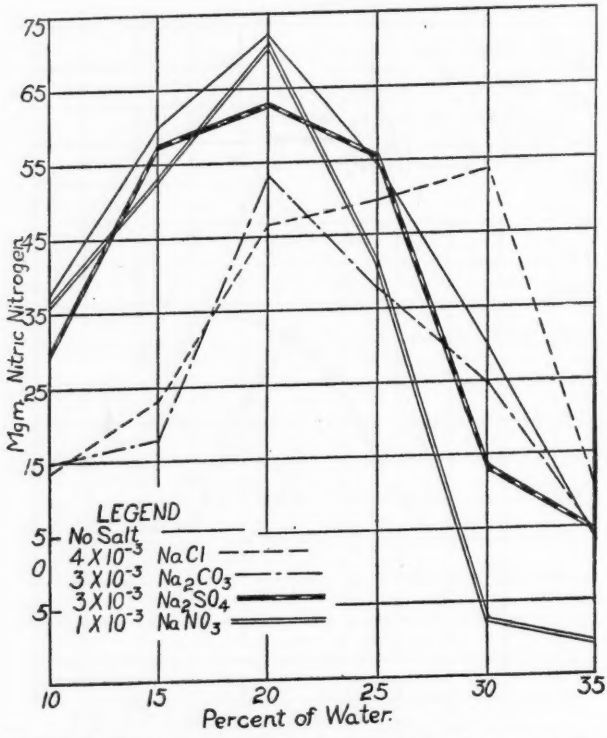

FIG. 9. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT SODIUM SALTS, AND VARYING PERCENTAGES OF WATER

applied to the air-dried soil. The soil reached its maximum nitrifying power when it received 20 per cent of water and lost it rapidly as the amount of water applied was increased. When the water-content reached 35 per cent nitrification practically ceased in all the tumblers except that receiving sodium chloride. In this there was an appreciable quantity of nitrates produced even at the highest water-content. Moreover, nitrification did not reach its maximum until this soil had received 30 per cent of water. Whether this was due to an actually increased nitrification or to a decreased synthetic

reaction is impossible to say. But inasmuch as the optimum moisture content for many of these changes was 30 per cent, it appeared probable that the causative factor was the latter. At a moisture-content of 30 or 35 per cent the nitrate-treated soil lost nitrates, but actual test showed that they did not lose nitrogen. In this case it was merely an acceleration of the synthetic reaction together with a slowing up of the nitrate produced. The proportion of nitrates found in the chloride-, carbonate-, and sulfate-treated soil increased

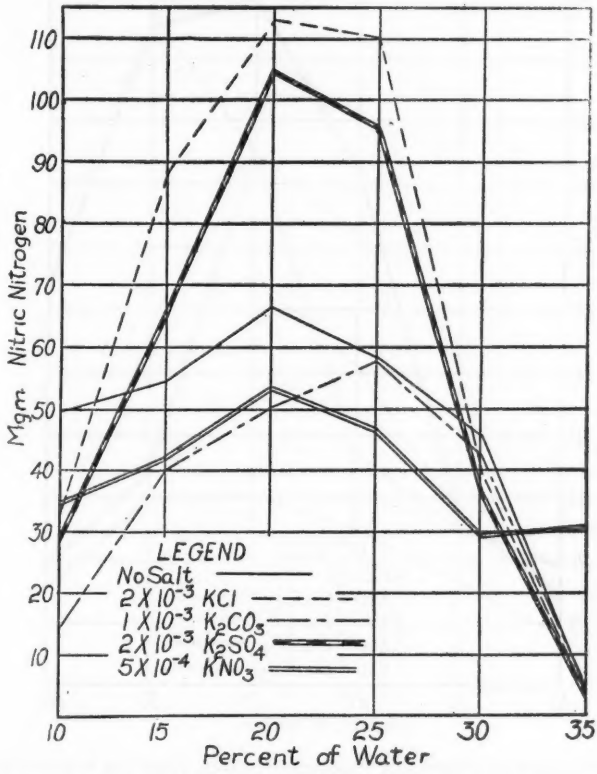

FIG. 10. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT POTASSIUM SALTS, AND VARYING PERCENTAGES OF WATER

as compared with the untreated soil, whereas that in nitrate-treated soil decreased with the increase of moisture-content.

This loss of nitrates did not appear in the case of potassium nitrate-treated soil (fig. 10) which lost no nitrates even when the soil contained 35 per cent of water. The potassium chloride-treated soil reached its maximum nitrifying efficiency when 25 per cent of water had been added to the soil,

whereas the untreated soil and those receiving the other potassium salts all reached their maximum activity at a moisture-content of 20 per cent of water. This was similar to the results obtained with the ammonifying series. The potassium chloride-treated soil required greater quantities of water for maximum ammonification than did the soils receiving the other salts. When the water content reached 35 per cent nitrification nearly ceased in all of the soils except that treated with potassium nitrate. The quantity of nitrates found

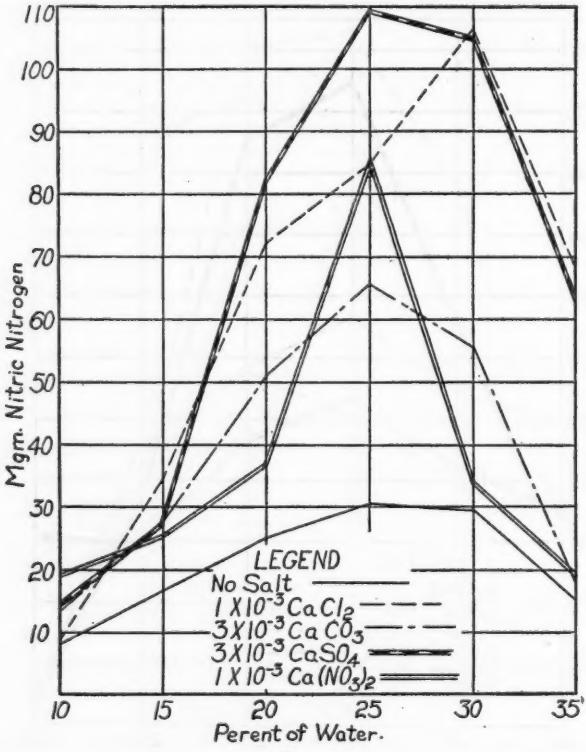

FIG. 11. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT SODIUM SALTS, AND VARYING PERCENTAGES OF WATER

in the chloride- and sulfate-treated soil increased relatively to the untreated soil as more water was applied, This increase was most pronounced at a content of 20 and 25 per cent of water. In soil containing 10 per cent of water both of these salts were toxic, but in soil containing 20 per cent of water there was found nearly twice as much nitric nitrogen as in the untreated soil. The treated and untreated soils produced practically the same quantities of nitric nitrogen at moisture-contents of 30 and 35 per cent. It was quite

evident from these results that the relative toxicity of these salts decreased as the water content of the soil increased, a condition to be looked for when the toxicity is an osmotic effect.

Soil treated with chloride of calcium, as was the case with the chloride of potassium and sodium, required more water for maximum nitrification than did those treated with the other salts (fig. 11). This would seem to indicate that the electro-negative ion is the controlling factor in the water re-

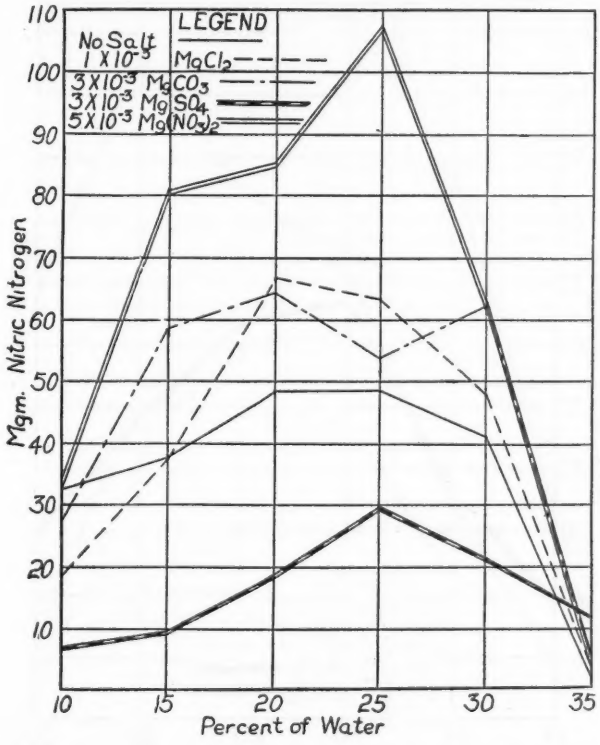

FIG. 12. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT MAGNESIUM SALTS, AND VARYING PERCENTAGES OF WATER

quirements of this class of organisms. The quantity of nitrates produced in the salt-treated soil was invariably higher than in the untreated soil, and this difference became greater in every case as the amount of water added to the soil was increased up to 25 per cent. This must have been due to a direct action of the salts upon the organism and not to a change in the calcium-magnesium ratio, for the same phenomenon appeared when magnesium salts were applied, (fig. 12). This soil already contained 7.41 per cent of

calcium oxide and 4.15 per cent of magnesium oxide, yet the addition of calcium and magnesium salts greatly increased the quantity of nitric nitrogen accumulated. This, however, may have been an apparent, not a real increase in nitrification, for all these salts decreased the quantity of ammonia found at unit time to a greater extent than they increased the nitrification. Hence, it is reasonable to conclude that the calcium and magnesium salts probably retard the action of the organisms found in the soil which utilizes nitrates.

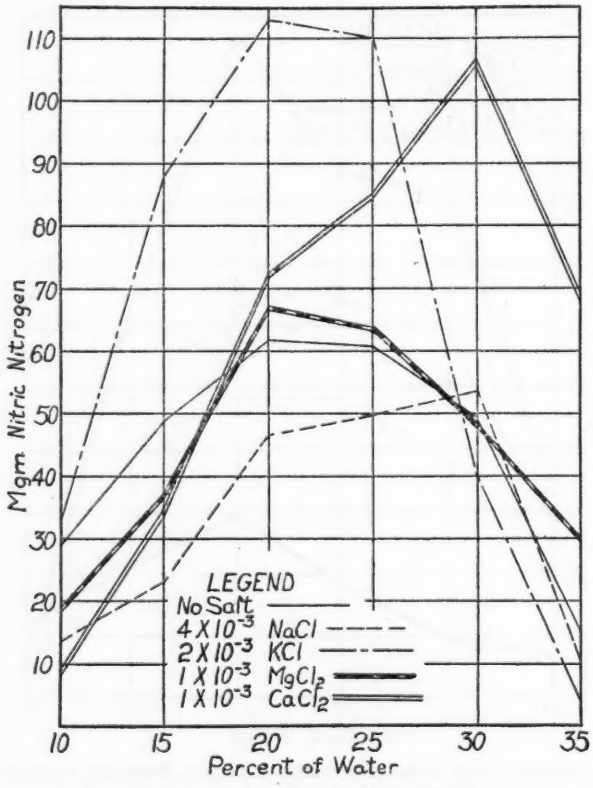

FIG. 13. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT CHLORIDES, AND VARYING PERCENTAGES OF WATER

The result of treating the soil with salts having a constant quantity of the electro-negative ion chlorine and a variable electro-positive ion are given in figure 13. Sodium chloride-and calcium chloride-treated soil reached their maximum nitrifying powers at a content of 30 per cent water, whereas potassium chloride-and magnesium chloride-treated soil reached their maximum at a content of 20 per cent water. This indicates a very marked influence on the part of the electro-positive ion. All of the salt-treated soils

at some water content were more efficient nitrifiers than the untreated soils. This is an important phenomenon, that a soil containing a certain salt may be toxic to nitrifying bacteria at one water-content but at a different water-content may become a favorable medium for these bacteria. This property is a characteristic of the various chlorides and is in keeping with what would be expected if the toxicity were due to osmotic influences.

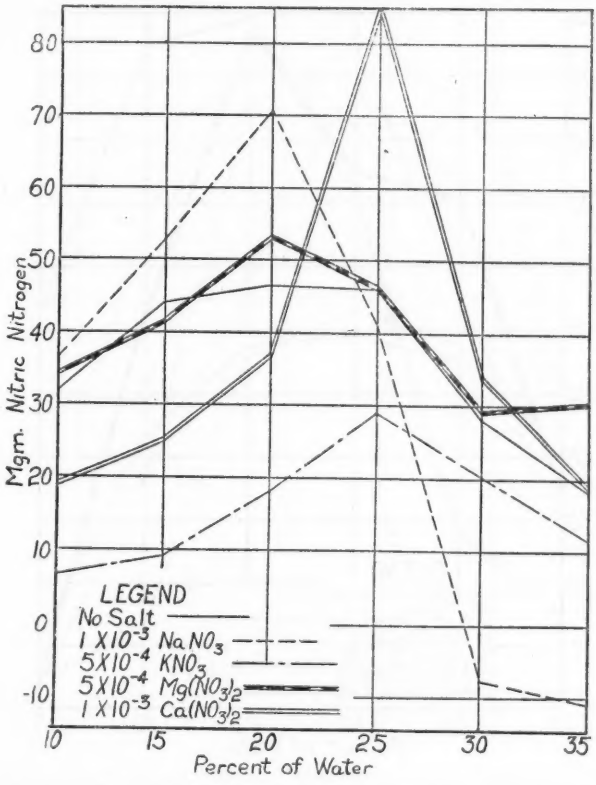

FIG. 14. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT NITRATES, AND VARYING PERCENTAGES OF WATER

This same phenomenon appeared in the case of the nitrates (fig. 14) and was very pronounced in soil which received calcium nitrate. When 1×10^{-3} mol of calcium nitrate were added to soil containing 10 per cent of water the nitrifying powers were decreased one-third, whereas this same quantity of calcium nitrate in soil containing 25 per cent of water nearly doubled the nitrification. Moreover, it is interesting to note that sodium nitrate was the only nitrate of those tested which, even in the presence of the highest content of

water, caused a loss of nitrates. It had repeatedly been our experience that treatment with sodium nitrates caused a disappearance of nitrates from soil, whereas potassium, calcium, and magnesium nitrate seldom caused a loss. Furthermore, we have experienced difficulty in inducing denitrification in our highly calcareous soils by the addition of nitrates, organic matter or water, either singly or in combination. At times there may be a disappearance of

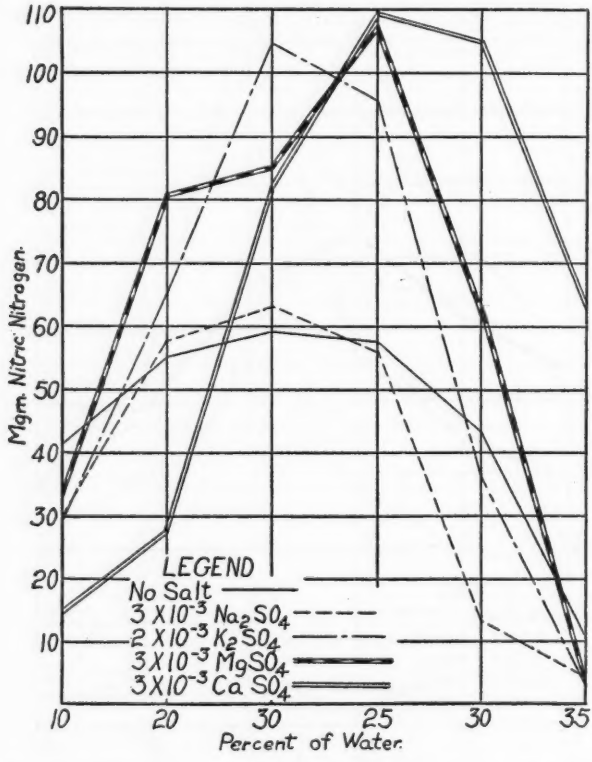

FIG. 15. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT SULFATES, AND VARYING PERCENTAGES OF WATER

nitrates, but an analysis of the soil for total nitrogen usually shows that it has been transformed into protein nitrogen.

This passage from a toxic to a stimulating medium by increased water content was very pronounced in the case of the sulfates (fig. 15). It is a well-known fact that in the spring when the water-content of the soil is high many plants can be started on alkali soil, but as the water evaporates the plants are killed. This is due in a great measure to the raising of salts from lower levels

by capillarity, but these results indicate that the mere removal of the water may play an important part in the killing of the plant. They indicate that the dilution of the salt content is important, as well as its removal by rains of winter and spring. This dilution influence was only slight in soil containing sodium sulfate but was very pronounced in soil containing potassium magnesium and calcium sulfate.

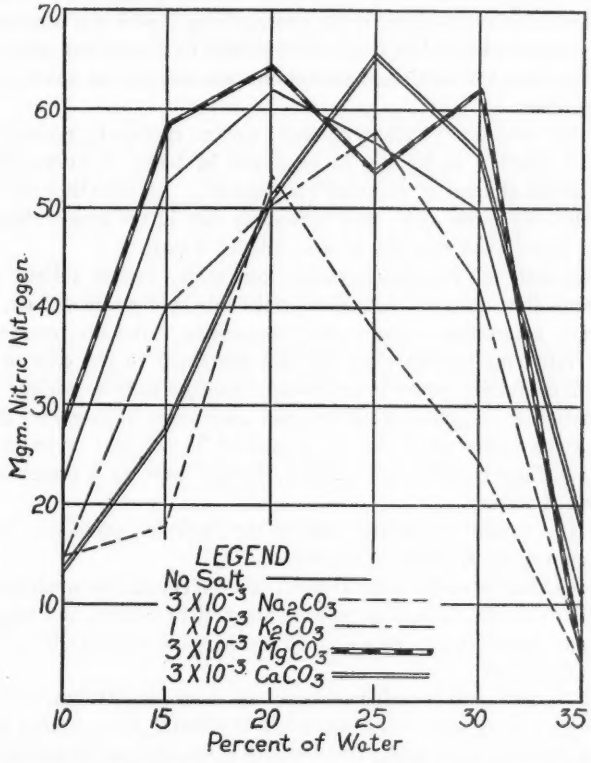

FIG. 16. CURVES OF NITRIC NITROGEN PRODUCTION IN SOIL, WITH AND WITHOUT CARBONATES, AND VARYING PERCENTAGES OF WATER

The influence of dilution by water was slight in soil treated with the carbonate (fig. 16). It did not appear at all in the case of sodium and potassium carbonate and was small with magnesium and calcium carbonate.

SUMMARY

The influence of the chlorides, nitrates, sulfates, and carbonates of sodium, potassium, magnesium, and calcium upon the moisture requirements of a

highly calcareous soil for bacterial activity was determined. The water-holding capacity of this soil (3) was 45 per cent.

Untreated soil, that receiving sodium carbonate, potassium carbonate, potassium nitrate, calcium carbonate, calcium sulfate, calcium nitrate, magnesium chloride and magnesium carbonate, had maximum ammonifying powers when the soil contained 30 per cent of water.

Soils containing sodium sulfate, sodium nitrate, potassium chloride and potassium sulfate possessed maximum ammonifying power at a water-content of 35 per cent, whereas when magnesium sulfate or magnesium nitrate were added to the same soil maximum ammonification occurred at a content of 25 per cent of water.

The relative toxicity of sodium chloride, sodium carbonate, potassium carbonate, and calcium carbonate, as measured in terms of ammonification, decreased as the amount of water added increased. All the other salts tested became relatively more toxic, thus indicating that in the case of these latter some other factor than osmotic pressure played a part.

Untreated soil, soil containing sodium carbonate, sodium sulfate, sodium nitrate, potassium chloride, potassium carbonate, potassium sulfate, potassium nitrate, magnesium chloride and magnesium carbonate, exerted their maximum nitrifying powers when the soil contained 20 per cent of water; whereas soil containing potassium carbonate, calcium sulfate, calcium nitrate, calcium carbonate, magnesium nitrate, and magnesium sulfate were at maximum nitrifying power when the soil contained 25 per cent of water. Soils containing sodium chloride and calcium chloride were at a maximum with 30 per cent of water.

The relative toxicity of various salts to the nitrifying organisms decreased as the amount of water added was increased.

The mere changing of the water content of a soil containing small quantities of potassium chloride, potassium sulfate, magnesium nitrate, and magnesium chloride may transform it from a toxic medium for nitrifiers to a strongly stimulating medium.

When 1×10^{-3} mol of sodium nitrate was added to 100 gm. of soil containing 35 per cent of water there was a loss of nitrates, but a similar quantity of calcium nitrate or even larger quantities of magnesium or potassium nitrate caused no loss of nitrates even in the presence of 35 per cent of water.

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EFFECT OF DIFFERENT REACTIONS ON THE GROWTH AND NODULE FORMATION OF SOYBEANS[1]

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Although it is well known that soil acidity and alkalinity have a marked effect on cultivated plants, especially certain legumes, yet, it has never been definitely determined at what reaction the legumes grow best and become inoculated most abundantly. Whether the favorable range of reaction is wide or narrow, and what this range is for each of the legumes are very important questions from both a practical and a scientific standpoint. In order to gain further information on these questions, the present investigation was undertaken.

The soybean was used in the beginning because of its adaptation to water cultures and because of its importance as a crop. Several different strains of soybean bacteria were studied. For comparison, some results with corn and cowpeas obtained under similar conditions are reported herein.

An exhaustive discussion of the literature on this subject does not seem necessary and only a brief review of some of the more important papers is given. The magnitude of the hydrogen- and hydroxyl- ion concentration of a number of soil solutions and suspensions have recently been determined by Gillespie (15), Sharp and Hoagland (35), Plummer (32), Truog (37), and others. These investigations have shown that soil solutions have a wide range of reactions, namely, from pH 3 to pH 9.

The importance of the effect of the soil reaction on the soil bacteria is well recognized. Gruzit (14) reported that an alkaline reaction was more favorable for the growth of the bacteria than an acid, or even neutral reaction. Salter (33) reported that red clover bacteria do best at a neutral or slightly acid reaction while the alfalfa bacteria do best at a slightly alkaline reaction. Fred and Loomis (11) reported that an alkaline reaction (pH 7.72) produced maximum growth of the alfalfa bacteria. Fred and Davenport (12) reported that a correlation exists between the acid resistance of the nodule bacteria and the

[1] Part I of a thesis submitted at the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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acid resistance of the higher plants. Bewley and Hutchinson (2) reported that some of the legume bacteria are either killed in definitely acid soils, or at least lose their activity, while Hiltner (21) claimed that liming has an injurious effect on lupine bacteria.

The sensitiveness of plants to the reaction of dilute solutions of acids,bases and salts was noted by Kahlenberg and True (26), Heald (20) and Loew (29). These investigators studied primarily the direct chemical effect of reaction on seedlings and not the influence on growth, and hence did not use balanced nutrient solutions. Because of this condition definite conclusions can not be drawn from their data regarding influence on growth. Their data show, however, that seedlings differ widely in their behavior toward reaction.

Cameron and Breazeale (4) reported that corn was much more acid tolerant than wheat or clover. Hartwell and Pember (18) claimed that some of the cereals are more sensitive to acidity than others but no statement was made as to the relative sensitiveness. Hoagland (23) reported that barley seedlings grew better in a slighty acid reaction than in a neutral or alkaline reaction. This was also reported by Salter and McIlvane (34) in working with corn, wheat, soybeans and alfalfa. The data of the latter indicate that alfalfa is more sensitive to acidity than corn, wheat or soybeans. Joffe (25) reported that alfalfa produced good growth in soil cultures which were made very acid (pH 3.8) by the addition of sulfuric acid. Hixon (22) noted that there was a difference in water content, organic matter, and total ash in the roots of 16-day-old wheat seedlings grown at different reactions.

Hartwell and Pember (18) and Dachnowski (10) claimed that the hydrogen ion was more toxic to plant growth than the hydroxyl ion. This question was investigated by Hoagland (23) who reported the opposite results with barley seedlings. These contradictory results are possibly due to different methods of determining the reactions. Whether total acidity or hydrogen ion concentration is used as a criterion for indicating reactions is of great importance. Other factors, e. g., kind of nutrient solution used and proper maintenance of the desired reaction, are also important.

The change in reaction of the nutrient solution in contact with growing plant roots was noticed by Hartwell and Pember (18) who reported that an acid solution tended to become alkaline. This was also reported by Breazeale and LeClerc (3) who claimed from their work with wheat seedlings that the change in reaction of the nutrient solution was due to the selective absorption of the ions by the plants. The buffer condition of the nutrient solution and the frequency of renewing the solution are no doubt important factors in maintaining a constant reaction. The change in reaction of the nutrient solution in contact with plant roots during 1-day periods was found by Hoagland (23) to be quite considerable provided the initial reaction was not favorable.

Salter and McIlvane's (34) results indicate that their nutrient solution remained fairly constant over a period of 4 days when in contact with growing plants. Duggar (9) concluded that, in general, an acid or an alkaline

solution tended toward neutrality when in contact with growing plants. However, this change depended on the nutrient solution used and the rate of plant growth. The most favorable reaction also varied with the kind of nutrient solution used.

In connection with the influence of reaction on plant growth, it is well to mention the toxic effects of soluble aluminum salts as reported by several investigators. Abbott, Conner and Smalley (1) reported that the toxic effect of aluminum nitrate was about the same as a solution of nitric acid of equal normality; and that water extracts from acid soils containing soluble aluminum salts were as toxic to plant growth as a nutrient solution containing an equal amount of aluminum salts. Hartwell and Pember (19) found that aluminum salts were considerably more toxic to barley seedlings than to rye seedlings. The toxic effect of acidity alone was about the same on both plants. They concluded that lime may precipitate the aluminum and thus be of value in this way as well as in neutralizing the acidity. Mirasol (30) reported that aluminum salts which could be extracted from the soil with a solution of potassium nitrate were probably the cause of the unproductiveness of three acid soils of Illinois. Conner (6) reported that much of the harmful effects of acidity of soils is due to soluble aluminum salts; and the presence of abundant lime or phosphate will prevent this harmful effect. He noted that some plants are more sensitive to aluminum salts than others. This condition was also noted by Hartwell.

Undoubtedly, in very acid soils aluminum, manganese and other toxic substances go into solution and produce injurious effects on cultivated plants.

The results of the various investigations may be summarized as follows: The range of reaction of different soils is sufficiently wide to give conditions of acidity and alkalinity in some cases which are unfavorable to bacteria and higher plants. The different legume bacteria vary in their behavior toward reaction, and the degree of the acid resistance appears to be in the same direction as that of the host plants. In the case of plants, there are some contradictory results, but, in general, they indicate that plants differ in their behavior toward reaction. This is in line with field observations. In studying a problem of this nature, there are a number of factors which are difficult to control and the conflicting results obtained by the different investigators are perhaps due in part to the difficulty of controlling the reactions, and in some cases to the use of unfavorable nutrient solutions.

In regard to the toxic effects of soluble aluminum and other substances in acid soils, it appears that plants vary in their behavior toward these toxic substances and that the harmful effects of acidity in some soils are due in part to soluble aluminum salts.

GROWTH OF DIFFERENT STRAINS OF SOYBEAN BACTERIA AT VARIED REACTIONS

Since some investigators (2) had noted that many of the legume bacteria are either killed, or, at least rendered inactive in acid soils, it was thought best, as

a preliminary experiment, to determine the critical hydrogen- ion concentration of the various strains of soybean bacteria in pure cultures and also to determine if there is any marked difference in behavior of these strains of bacteria at different reactions. By strains of bacteria, is meant a pure culture isolated from a known variety of soybeans.

Twenty-one[2] strains of soybean bacteria were grown on mannit, on sucrose and on soil-extract agars. The mannit and sucrose agars were prepared according to Ashby's formula as follows:

Mannit or sucrose	15.0 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
KH_2PO_4	0.2 gm.
NaCl	0.2 gm.
$CaSO_4 \cdot 2H_2O$	0.1 gm.
Agar	15.0 gm.
Distilled water	1000.0 cc.

The soil extract agar was prepared by diluting 100 cc. of soil extract from a silt loam soil to 1000 cc. and adding 15 gm. each of mannit and agar. The range of reaction used was from pH 3.3 to pH 10 with the sucrose agar and from pH 3.3 to pH 7 with the mannit and soil extract agars. The desired reactions were obtained by adding varied amounts of sterile sulfuric acid and sodium hydroxide, as the case required, before the media were allowed to solidify in slants. The Clark and Lub's method for colorimetric determination of the reactions of solutions was employed. Each culture was inoculated with one drop of suspension containing the bacteria. Triplicates of each reaction were used in all cases. The cultures were incubated 15 to 20 days at 28°C. before final results were recorded.

In general the mannit and soil extract agars showed a more vigorous growth than the sucrose agar, although the three media showed only minor differences in critical pH for the various strains of bacteria. There were small differences in growth of bacteria between the reactions pH 7 and pH 6.5 but an increase in hydrogen-ion concentration from pH 6.5 to the critical concentration produced a gradual decrease in growth with each strain of bacteria. Eight strains of the bacteria were grown in the alkaline range, all of which grew at pH 10. The maximum growth of the eight strains grown in the alkaline range took place at reaction of about 7.6. Table 1 gives the critical hydrogen-ion concentration for the different strains of soybean bacteria studied. By critical hydrogen-ion concentration is meant the reaction at which the bacteria do not produce any visible growth during the 15 days after inoculation. It will be noted that there is not a great difference in the critical hydrogen-ion concentration for the various strains of soybean bacteria.

[2] Fourteen of the different strains of soybean bacteria studied were furnished through the kindness of the Bureau of Plant Industry, of the United States Department of Agriculture One strain was furnished through the kindness of Dr. A. L. Whiting, University of Illinois.

TABLE 1

The critical hydrogen-ion concentration for soybean bacteria on mannit, sucrose, and soil extract agar

STRAIN OF BACTERIA	NUMBER	CRITICAL pH VALUE
Arlington	118	4.2
Barchet	293	4.2
Barchet	218	4.6
Chinese	271	4.6
Cloud	270	4.2
Cornell	150	4.5
Haberlandt	210	4.0
Haberlandt	334	4.0
Hobta	337	4.0
Manchu	312	4.7
Manchu, Wisconsin Experiment Station	154	4.6
Medium early, Illinois Experiment Station		4.0
Mammoth yellow	338	4.2
Pingsu	275	4.2
Roosevelt	181	4.6
Roosevelt	187	4.0
Soybean	152	4.6
Taha	233	4.4
Tokio	256	4.6

PLANT CULTURES

Methods

The plants were grown in solution and sand cultures in the greenhouse from March 1 to July 25. The culture vessels used were 500 and 600-cc. percolators. These were provided with glass tubes and pinch-cocks at the bottom in order that the solutions might be easily removed without disturbing the plants. In the case of sand cultures, the percolators were supported by iron ring stands and wrapped in heavy brown paper to exclude the light. Those containing solution cultures of soybeans were placed in boxes and surrounded with moist sawdust to exclude light and prevent rapid changes of temperature. The glass tubes extended through holes in the boxes in order that the solutions might be removed readily. A few solution cultures of corn and cowpeas were also set up. For holding these percolators, circular holes were cut of such size in the top of a greenhouse bench that the percolators passed through to the rim around the mouth which acted as a support. The light was excluded from beneath the bench.

The most desirable nutrient solution for this type of investigation was unknown at the start. A modified form of Shive's three-salt solution was first used with the soybeans. The modification consisted in reducing the amount of $Ca(NO_3)_2$ to one-fourth that recommended by Shive, and adding an equal amount of $CaCl_2$. This made a total reduction of calcium to about one-half

that in the regular solution. The chloride was substituted for the nitrate, since large quantities of nitrates are known to hinder nodule formation. The reduction of calcium was made to reduce the precipitation of $Ca_3(PO_4)_2$ in the alkaline solutions. This modified solution proved unfavorable for nodule formation of soybeans in solution cultures. Shive's regular solution with varied amounts of nitrates was then tried, but again no nodules developed. Several other nutrient solutions, namely, Pfeffer's, Hopkins-Pettit's, Crone's, and also Mendota Lake water were tried with the result that only in Crone's nutrient solution and Mendota Lake water were nodules produced. Crone's solution being the most satisfactory, was chosen with slight modification for the work reported herein. The modification will be given later.

The reactions used in all cases were approximately pH 3, 4, 5, 6, 7, 8, 9 and 10. For specific reactions, see table 6. The Clark and Lub's method for colorimetric determination of reaction of solutions was employed in making the adjustments. The standards were checked at intervals with the hydrogen electrode. In order to maintain the reaction of the nutrient solution, in contact with plant roots, as constant as possible the solutions were renewed daily.

For the sand cultures, 20-mesh (Ottawa silica) sand was used. This was first thoroughly washed with distilled water. The sand was then placed in the percolators and washed with the respective solutions until the reaction remained constant on passing through the sand. The solution in sand cultures as in solution cultures were renewed daily. This was done by means of suction as sugested by McCall (31). The percolators were ideally adapted for changing of solutions.

The seeds were germinated in clean quartz sand and allowed to grow 3 to 5 days before being transferred to the percolators. Two seedlings were grown in each percolator both in sand and solution cultures. The seedlings, in case of the solution cultures, were held in place by means of paraffined corks and plugs of cotton. In order that the seedlings would not be subjected to a great change in reaction at once when being transferred to the percolators, the acid and alkaline reactions of the nutrient solution were brought to the desired points gradually over a period of 2 days. The solutions were inoculated 3 days after the seedlings had been transferred to the percolators. This was done by placing the inoculum in the nutrient solutions at the time the solutions were renewed. The plant cultures were grown for 25 to 35 days.

Growth of soybeans in Shive's nutrient solution at different reactions

As previously stated, a modified form of Shive's nutrient solution was first used in both sand and solution cultures. It was prepared from the following stock solutions in which the amounts of salts are indicated on the anhydrous basis.

(a) $CaCl_2$ ..... 50.0 gm.
$Ca(NO_3)_2$ ..... 50.0 gm.
Distilled water ..... 500.0 cc.

(b) $MgSO_4$ ..... 50.0 gm.
Distilled water ..... 500.0 cc.

(c) K_2HPO_4 ..... 50.0 gm.
Distilled water ..... 500.0 cc.

(d) $FeCl_3$ ..... 2.5 gm.
Distilled water ..... 300.0 cc.

Ten cubic centimeters of (a), (b), (c), and ten drops of (d) were added to 4 liters of distilled water. Dilute sulfuric acid or sodium hydroxide was added to portions of this solution until the desired reactions were obtained. The general method of procedure already outlined was followed. The plants were allowed to remain in the solution 24 days from time of inoculation.

TABLE 2

Growth and inoculation of soybeans in sand cultures with Shive's nutrient solution at different reactions

REACTION OF CULTURE	NODULES PER PLANT	PLANT DEVELOPMENT
pH		
3.2	0	Tops fair Roots dark and stubby
4.2	5	Roots better than at pH 3.2
5.0	12	Tops good Roots slightly dark
6.0	35	Tops good Roots good
6.9	49	Tops good Roots good
8.0	11	Tops good Roots slightly brown
9.0	2	Tops small Roots very brown
9.9	0	Very poor

The results of all plants in all experiments at different reactions will be referred to as at pH 3, 4, 5, 6, 7, 8, 9, and 10 although the actual pH value may have been slightly more or less. For specific reaction with Shive's nutrient solution, see table 2.

Only two nodules developed in Shive's nutrient solution and these appeared at pH 8. A poor and injured condition of the plants indicated that something was wrong with the nutrient solution. This may have been due to the presence of toxic impurities in the salts used. Plants at pH 3, 4 and 10 were almost

dead. Sand cultures of soybeans with Shive's nutrient solution were also carried on according to the regular method already described. The results of growth and inoculation with these are given in table 2. The best growth and inoculation were obtained at pH 6 and 7. In contrast to plants grown in solution cultures, the plants grown in sand cultures showed no injurious effect other than that due to reaction. The plants at pH 3, 4 and 10 had very dark roots and small tops. In general, a considerable increase in acidity or alkalinity caused a decrease in plant growth. Perhaps the absence of injurious effects in the sand cultures was due to adsorption of toxic substances from the solution by the sand.

The effect of nitrates on nodule formation in Shive's nutrient solution

Since practically no nodules developed in solution cultures with Shive's solution, it was thought that possibly the presence of nitrates prohibited inoculation. To determine the influence of nitrates on nodule formation, soybeans were grown in Shive's nutrient solutions containing 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ times the usual amount of nitrates recommended by Shive, and also in one solution entirely without nitrates.

For culture vessels, 500-cc. wide-mouthed bottles were used, and two soybean seedlings which had been germinated in clean quartz sand were placed in each. The seedlings were held in place with paraffined corks and plugs of cotton. The solutions were inoculated and renewed weekly. The plants were allowed to grow for four weeks, during which time not a single plant in any of the solutions developed any nodules. All the plants had yellowish leaves and somewhat dark and stubby roots indicating that the solution was toxic.

Influence of different nutrient solutions on nodule formation of soybeans

Since all attempts to get nodule formation on soybeans in solution cultures had practically failed, it was decided to determine the effects of different nutrient solutions on inoculation of soybeans. In addition to Shive's solution, Hopkin-Pettit's, Pfeffer's and Crone's solution, and also Mendota Lake water were used. The cultures were carried on in exactly the same manner as the previous ones. At the end of 4 weeks, the plants in Crone's nutrient solution and Mendota Lake water had developed a goodly number of nodules and the plant growth was healthy. Those in Shive's nutrient solution had developed only two nodules and the roots were dark and stubby, and those in the other two solutions did not develop any nodules at all. Their leaves were also yellowish and the roots dark. Plate 1 shows the root development and nodule formation in the different solutions.

Growth and inoculation of soybeans in Crone's nutrient solution at different reactions

The principal experiment on the influence of reaction on growth and inoculation of soybeans was next started with Crone's nutrient solution. The buffer condition of this solution was, however, unsatisfactory in the alkaline range and for this reason several different substances, namely, di-basic sodium phosphate, di-basic potassium phosphate, sodium glycero phosphate, and sodium carbonate and bicarbonate were added and tested for their buffer effect, and also influence on nodule formation. Of these, sodium carbonate at the rate of $\frac{3}{4}$ gm. per liter proved to be the most satisfactory, and hence was used. The salts for Crone's solution were ground to a fine powder and thoroughly mixed in the following proportions.

	gm.
KCl	100
$CaSO_4 \cdot 2H_2O$	25
$MgSO_4 \cdot 7H_2O$	25
$Ca_3(PO_4)_2$	25
$FePO_4$	25

For the solution, 12 gm. of this mixture were added to 8 liters of distilled water. The mixture was well shaken with the water and left to stand for one day, at end of which time the solution was filtered to remove the insoluble materials. The acid reactions were obtained by adding varied amounts of sulfuric acid. The alkaline reactions were obtained by first adding the sodium carbonate and then acid until the desired reaction existed.

The plants were grown in percolators according to the general method already outlined. Good plant growth took place at the favorable reactions. In all cases duplicates agreed as to nodule formation and root development. Plate 2 serves to indicate the relative size of plants and nodules per plants in solution cultures. It will be noted from plates 3 to 10, inclusive, that plants at pH 3, 4 and 10 had no nodules. Plants at pH 4.5 which is not given in figure 2 developed a few nodules, but at this reaction the roots were dark and stubby. Plants at pH 5 had fair inoculation. The tops of the plants at this reaction were as tall as those at pH 6, but were yellow and much less vigorous. Plants at pH 6 and 7 were decidedly the best, both as to growth and inoculation. Those at pH 6 were perhaps a trifle better than those at pH 7. Plants at pH 8 had some nodules but the roots were slightly brown. The roots at pH 9 and 10 were still browner and did not become inoculated. It should be noted that plants at pH 9 were a little taller than at pH 8, but of a very poor color. In all other cultures at pH 8 the plants were better than at pH 9. Table 3 gives dry weight of plants and number of nodules per plant.

The experiment just described was repeated using sand cultures according to general method already given. The same solution and reactions, were used as in the previous experiment. The extent of nodule formation was about the same as that in the solution cultures. The limits of nodule forma-

tion were pH 4 and 8. There was less injury from an unfavorable reaction at pH 3, 4, 5, 9 and 10 than in the solution cultures at these reactions. This was perhaps due to a smaller amount of nutrient solution actually in contact with the plant roots in the sand cultures than in the solution cultures, and hence a lesser amount of acid or alkali, making it easier for the plants to change the actual reaction to a more favorable one. Plate 13 gives the comparative growth in sand cultures.

TABLE 3

Growth and inoculation of soybeans in Crone's nutrient solution at different reactions

REACTION	DRY WEIGHT PER PLANT	NODULES PER PLANT	PLANT DEVELOPMENT
pH	*gm.*		
3.30	0.24	0	Tops dead Roots poor
3.97	0.40	0	Tops about dead Roots poor
4.95	1.00	30	Tops fair Roots dark and stubbed
6.50	1.42	77	Tops good Roots good
7.40	1.42	68	Tops good Roots good
8.20	1.15	21	Tops good Roots slightly brown
8.70	1.00	3	Tops fair Roots brown
9.60	0.60	0	Tops poor and yellow Roots very brown

Comparative growth of soybeans, corn and cowpeas at different reactions

For a comparison with the soybean, corn and cowpeas were grown in both solution and sand cultures with Crone's solution in exactly the same manner as the previous experiment with soybeans, except that nitrates instead of chlorides were used in the solutions for corn.

Plates 11, 12 and 14 serve to indicate the general growth which the corn and cowpeas made. The corn in the alkaline range of the solution cultures did not make good growth and the leaves were yellow. This was perhaps due to the lack of iron in solution, which was caused by precipitation under the alkaline conditions. The corn in the sand cultures grew better than in solution cultures and none of it became yellow. Corn plants at pH 5 in the sand cultures were inferior to the other plants at the beginning of the experiment and never grew as well as plants at pH 4. The maximum growth of corn took place at pH 6 and 7 in the sand cultures, and at pH 5 and 6 in the solution cultures. Plate 15 indicates that corn can grow at a considerably more

acid reaction than the cowpeas and soybeans. Apparently, as indicated in table 4 cowpeas have a wider range of inoculation than the soybeans. The corn was not grown in duplicate cultures and hence any conclusion with corn must be tentative.

TABLE 4

Growth and inoculation of cowpeas in sand cultures at different reactions

REACTION	NODULES PER PLANT	PLANT DEVELOPMENT
pH		
3.3	0	Tops fair Roots stubby and brown
3.97	20	Tops fair Roots slightly dark
4.95	22	Tops and roots good
6.5	32	Good plants, roots, and tops
7.4	33	Good plants, roots, and tops
8.2	26	Tops good Roots slightly brown
8.7	19	Tops fair Roots brown
9.6	14	Tops fair Roots very brown

The influence of the reaction of the culture medium on the reaction of the plant juices

The influence of the reaction of the culture medium on the reaction of the plant juices has been investigated by several workers (17, 38); and it was thought desirable to obtain additional information along this line. The reaction of the juices of the roots and leaves of some of the plants grown in the previous experiments was determined electrometrically by the method de-

TABLE 5

The reaction of the juices of plants grown in media of different reactions

REACTION OF MEDIA	SOYBEANS		COWPEAS		CORN	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
3.30	5.60	4.68	5.33	4.89	5.19	4.99
3.97	5.90	5.09	5.37	5.37	5.20	5.46
4.95	6.08	5.29	5.38	5.77	5.18	5.55
6.50	6.11	5.61	5.41	5.95	5.21	5.71
7.40	6.12	5.75	5.47	6.07	5.20	5.90
8.20	6.11	5.85	5.50	6.14	5.19	5.90
8.70	6.14	6.29	5.53	6.25	5.21	6.10
9.65	6.16	7.12	5.46	6.58	5.11	6.32

scribed by Clevenger (8) and Haas (17). The results are recorded in table 5. In general, the reactions of the plant juices as had previously been suggested (38) became more acid as the culture medium rose in acidity. The corn tops, however, showed no appreciable change. The reactions of the root juices were more nearly like those of the culture medium than were those of the leaves.

DISCUSSION

In making a comparison of the data presented, it should be clearly understood that pH 3 signifies an acidity ten times greater than pH 4, and pH 4 ten times greater than pH 5, etc. Hence, a small change in pH means a great difference in hydrogen-ion concentration. It will be noted from the data that the most favorable reaction for plant growth and nodule formation with soybean was from pH 6 to 7, which is a condition of slight acidity. This fact

TABLE 6

Change in reaction of Crone's nutrient solution in contact with soybean roots for 24 hours, as determined by the hydrogen electrode

CULTURE NUMBER	INITIAL REACTION	FINAL REACTION
	pH	*pH*
3	3.30	3.30
4	3.95	4.00
5	4.80	5.10
6	6.90	6.10
7	7.80	7.00
8	8.70	7.90
9	9.50	8.80
10	9.70	9.40

was indicated more clearly in the solution cultures than in the sand culture. The reactions at which maximum growth took place agrees with those reported by Hoagland (23) and Salter and McIlvane (34). Corn grew at a much stronger acidity than soybeans. This conforms with field observations.

The unsatisfactory results obtained in solution cultures with the modified form of Shive's nutrient solution are not readily explained since the plants grew well and became inoculated fairly well in the sand cultures. It was thought that possibly the salts used contained toxic substances which the sand adsorbed and thus prevented their toxic effect on the plants. In order to test this possibility some of the solution was thoroughly shaken with activated charcoal before being used. This appreciably lessened the injurious effects on the roots, but still no nodules appeared. Wilson (39) failed to obtain any nodules with soybeans in solution cultures using Pfeffer's nutrient solution even after varying the amount of nitrates. This same thing was found to be true with Shive's nutrient solution in the present investigation.

German and Didlake (13) reported inoculation of soybeans in solution cultures, using a nitrogen free commercial fertilizer for the nutrient salts. The writer believes that the unsatisfactory results obtained with Shive's nutrient solution in solution cultures were possibly due to toxic impurities in the salts used.

Crone's nutrient solution was not entirely satisfactory in the alkaline range because of the difficulty of maintaining a constant reaction when in contact with growing plants. This was even true to some extent after the addition of sodium carbonate as is indicated in table 6. The acid range was much more constant than the alkaline range, although it changed some. This same difficulty exists, of course, to some extent with all nutrient solutions, especially if the plants grow well in them.

The carbon dioxide excreted by the plant roots and that absorbed from the atmosphere were no doubt large factors in changing the reaction in the alkaline range.

It was found in the present investigation that when sodium glycero phosphate was added to Crone's solution the reaction remained almost constant for a daily period, but no nodules were formed in solution cultures. The addition of dibasic potassium phosphate to the alkaline range of Crone's solution was also found to hold the reaction of the solution fairly constant for daily periods, but nodule formation was again poor. In general, the reaction of a solution which was fairly favorable to plant growth so that considerable growth took place, would not remain constant very long in contact with the growing plants, unless the initial reaction of the solution was exactly the one most favorable for the plants. The change in reaction of the solution due to growing plants was always in the direction of a more favorable reaction. Salter and McIlvane's data (34) indicate that the reaction of their nutrient solution in contact with growing plants remained nearly constant during four-day periods. This was possibly due to the slow rate of growth of their plants.

The data show that pure cultures of soybean bacteria are able to grow at an acid reaction almost as great as that of the host plant. These results are not exactly in line with those of Bewley and Hutchinson (2) who reported that definitely acid soils would finally kill the nodule bacteria of lupines, red clover, and broad bean. However, they did not state the exact degree of acidity of the soil used and hence it is possible that they worked with very strongly acid soils. It will be seen from tables 2 and 3 that the best inoculation took place at a slightly acid to neutral reaction, although some inoculation took place at a reaction of pH 5 and even pH 4. The information secured thus far does not indicate that the critical hydrogen-ion concentration of pure cultures of the soybean bacteria is different from what it is in the soil.

The results show distinctly that the reaction of the media in which the soybeans are grown has a direct influence on growth and inoculation. The reactions which produced injury and poor inoculation were within the range of reactions of actual soil solutions and suspensions as reported by Gillespie

(15) Sharp and Hoagland (35), Plummer (32) Truog (37) and others. Different plants vary considerably in their ability to grow at reactions of pH 4, 5, and 6. Thus it will be seen that a proper adjustment of the reaction of the soil for different plants is of prime importance for the best growth and inoculation.

The reaction of the media in which the plants are grown often has a direct influence on the reaction of the plant juices. Further studies on the influence of reaction on legumes and legume bacteria are in progress.

SUMMARY

A study was made of the influence of acidity and alkalinity on growth and inoculation of soybeans in solution and sand cultures. The plants were grown in 500- and 600-cc. percolators. A modified form of Shive's nutrient solution was used at first, but with unsatisfactory results in solution cultures. Three other nutrient solutions were tested for influence on inoculation in solution cultures. Of these, Crone's nutrient solution proved to be the most satisfactory, and was selected with slight modification for the principal work reported herein. The reactions of the solutions were adjusted by adding varied amounts of acid or alkali as the case required. The reactions were kept as constant as possible by renewing the solutions daily. The old solutions were removed from the sand with suction before the new solutions were added. The cultures were allowed to grow from 25 to 35 days after inoculation. A few cultures of corn and cowpeas were grown under similar conditions to that of the soybean for comparison. Twenty-one different strains of soybean bacteria were grown on pure cultures of different reactions in order to compare the critical hydrogen-ion concentration of the soybean bacteria with that of the host plant.

1. Shive's nutrient solution was favorable for growth and inoculation of soybeans in sand cultures, but not in solution cultures. It seems possible that there were toxic impurities in the nutrient salts used which were adsorbed by the sand.

2. Crone's nutrient solution was favorable for growth and inoculation in both solution and sand cultures. The alkaline range of this solution has a very poor buffer action, which is improved markedly by the addition of $\frac{3}{4}$ gm. of sodium carbonate per liter.

3. The reaction of the nutrient solution in contact with growing plants does not remain constant very long, unless the initial reaction of the solution is the most favorable one for the plants. The rate of change in reaction is greater in the alkaline range than in the acid range. Plants growing rapidly apparently influence this rate of change more than slow growing plants.

4. The most favorable reaction for growth and inoculation of soybeans was pH 6.5. The limits for which inoculation took place were about pH 4.6 and 8. The limits for growth of soybeans were about pH 3.9 and 9.6. Reactions pH 4.95 and 8.2 are injurious to the growth of soybeans, but do not entirely prevent inoculation.

5. The hydrogen-ion concentrations which were markedly injurious to plant growth and inoculation in this investigation were not any greater and in some cases considerably less than the hydrogen-ion concentration of very acid soil solutions and suspensions as reported by recent investigators.

6. The critical hydrogen-ion concentration for nodule formation of soybeans was slightly less than that for plant growth. The different strains of soybean bacteria showed small differences in regard to critical hydrogen-ion concentration.

7. Corn grew at a greater acidity and alkalinity than the soybean or cowpea. The cowpea apparently has a greater range of reaction at which nodules are formed than the soybean.

8. The reaction of plant juices varied with the degree of acidity or alkalinity at which the plants were grown, except in the case of the juice of the corn leaves which showed little change. The juices of the roots followed the reaction of the media more closely than did the juices of the leaves.

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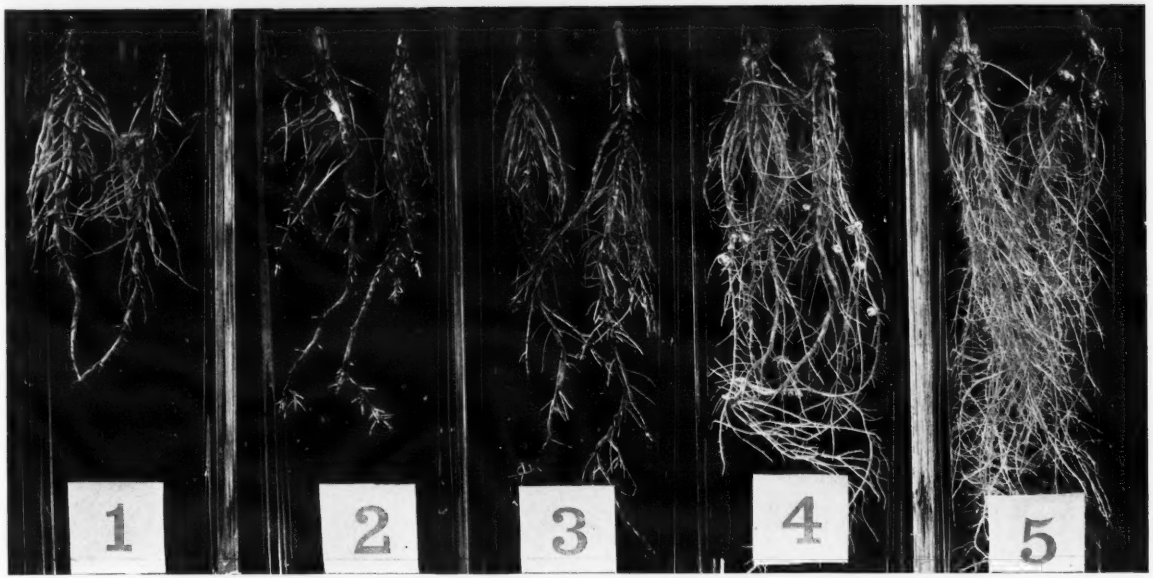
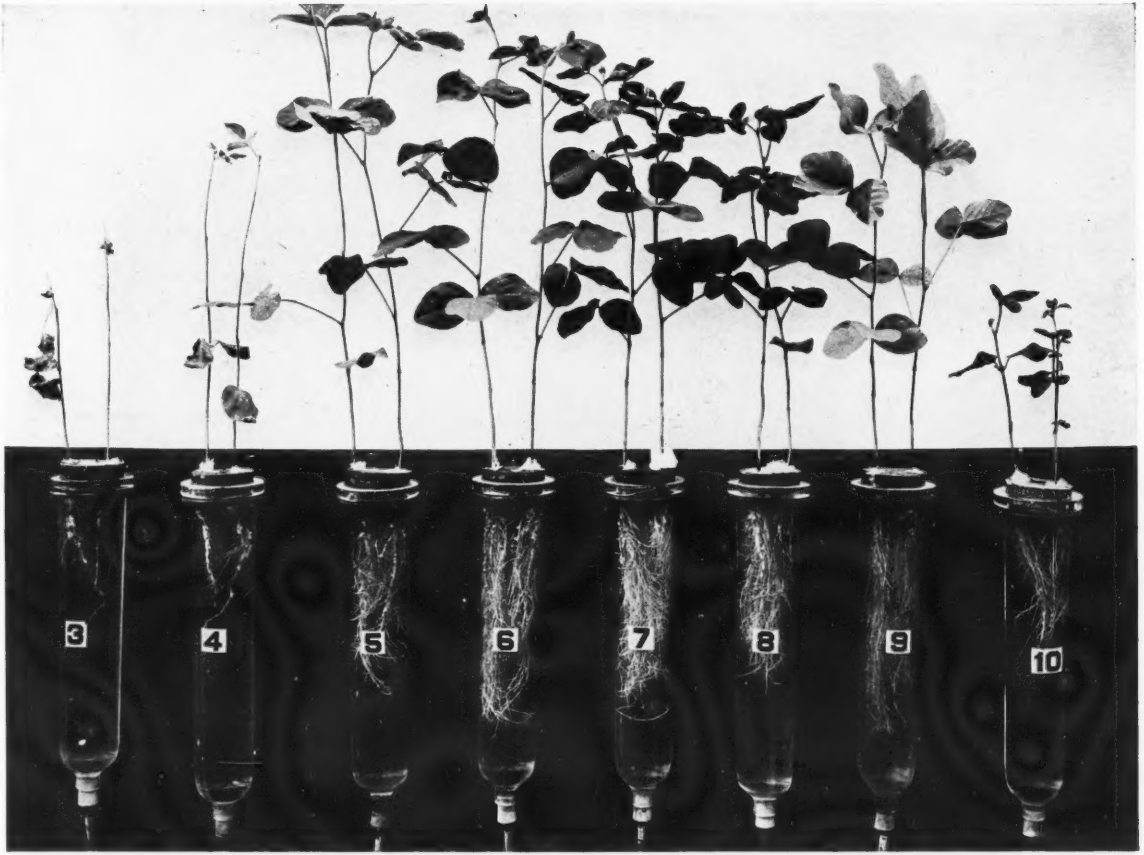


Shive's solution | Hopkin-Pettit's solution | Pfeffer's solution | Crone's solution | Mendota lake water

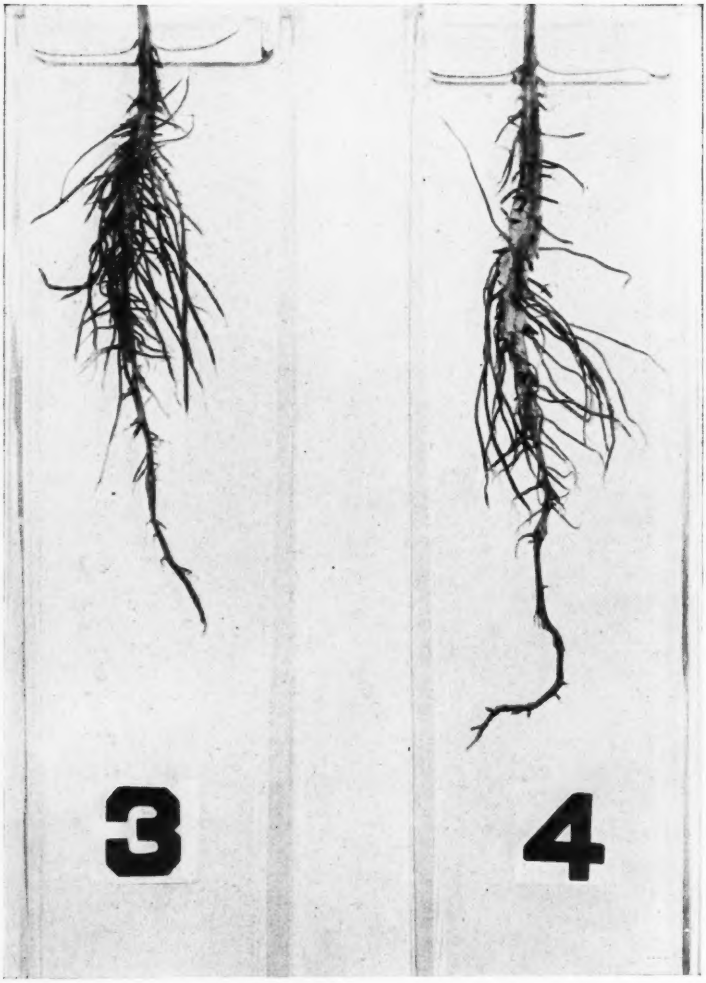
THE ROOT GROWTH AND INOCULATION OF SOYBEANS IN DIFFERENT NUTRIENT SOLUTIONS

EFFECT OF REACTION ON NODULE FORMATION

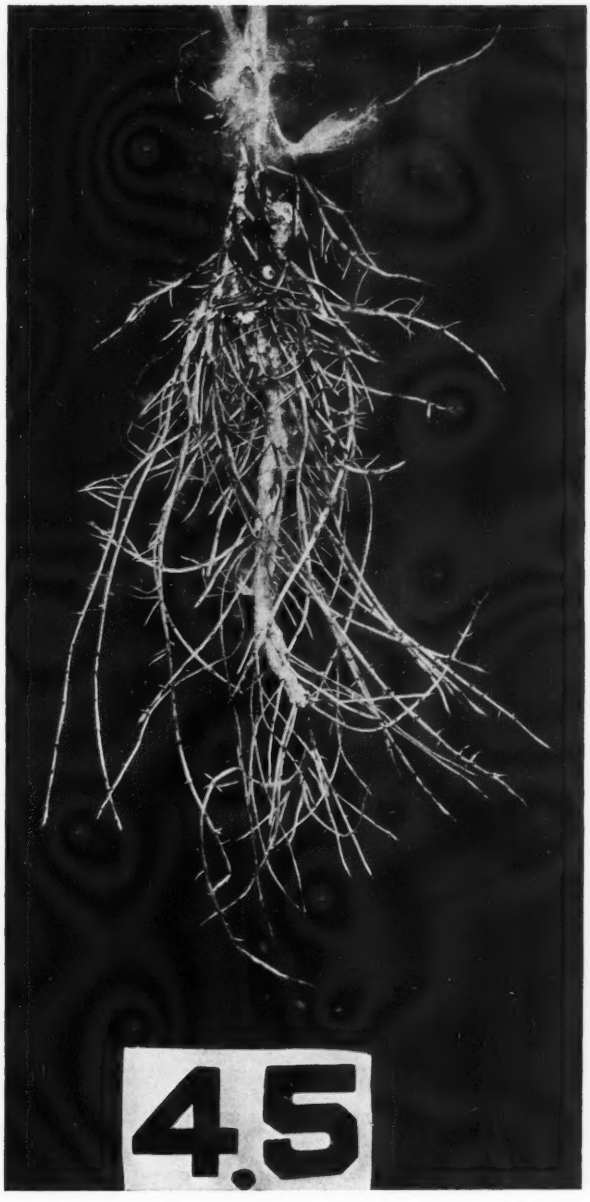
PLATE 1



SOYBEANS GROWN IN SOLUTION CULTURES OF DIFFERENT REACTIONS. THE NUMBERS REPRESENT THE APPROXIMATE pH VALUES



SOYBEAN ROOTS GROWN IN SOLUTION CULTURES WITH pH VALUES OF APPROXIMATELY 3 AND 4



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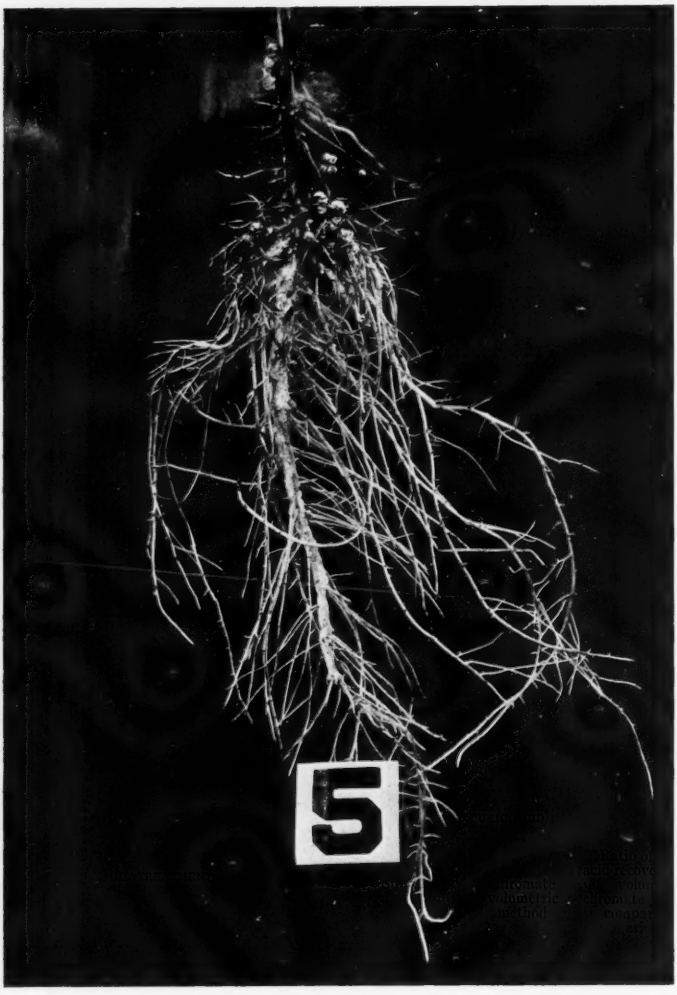
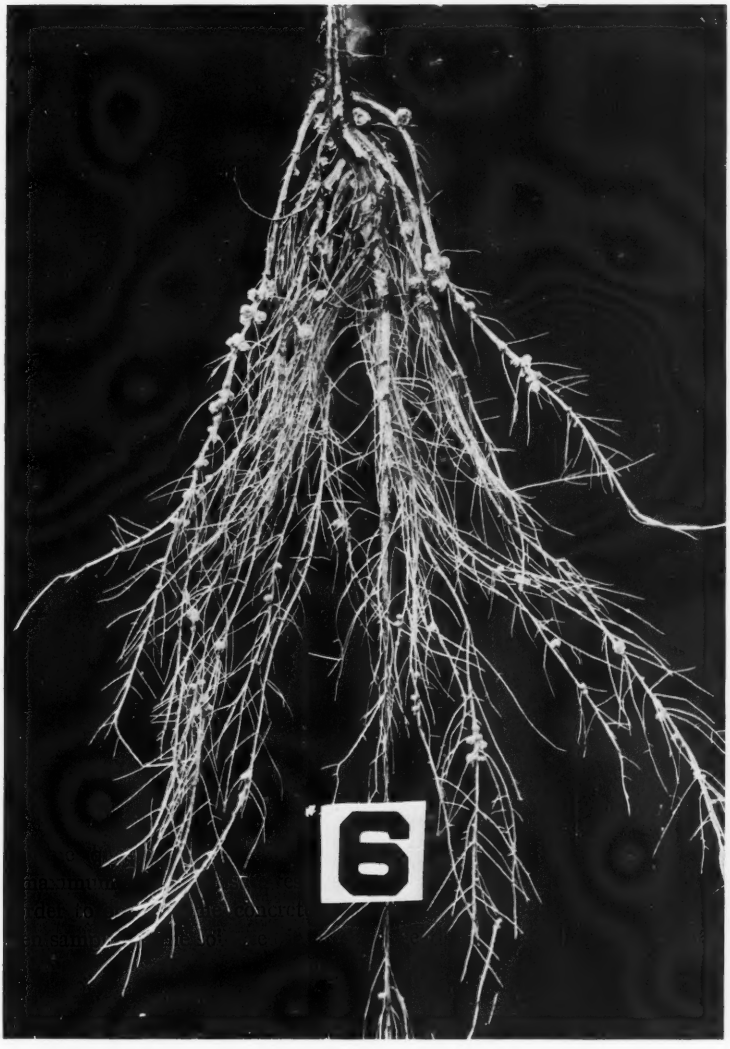


SOYBEAN ROOTS GROWN IN SOLUTION CULTURE WITH pH VALUE OF APPROXIMATELY 5



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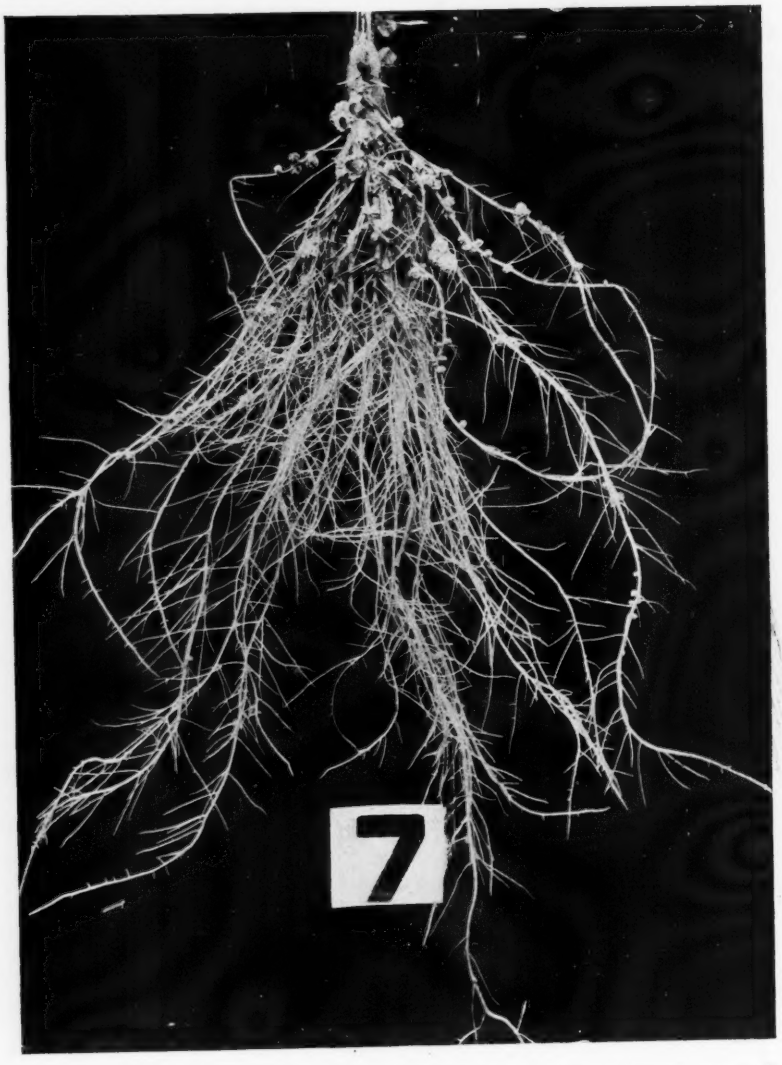

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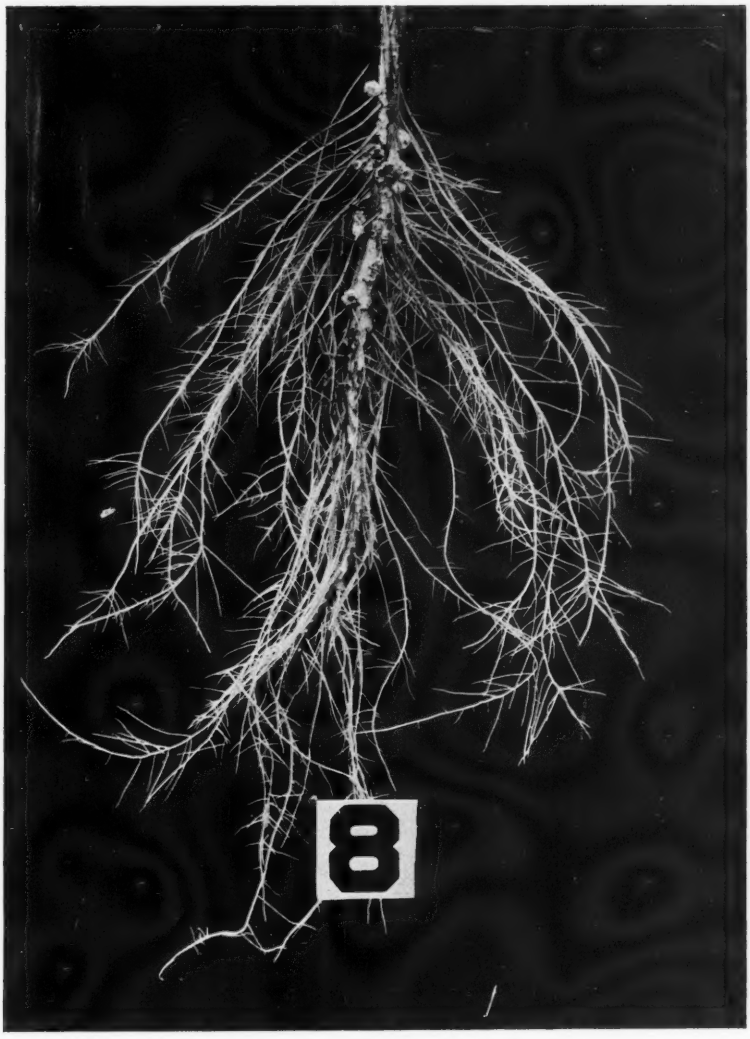
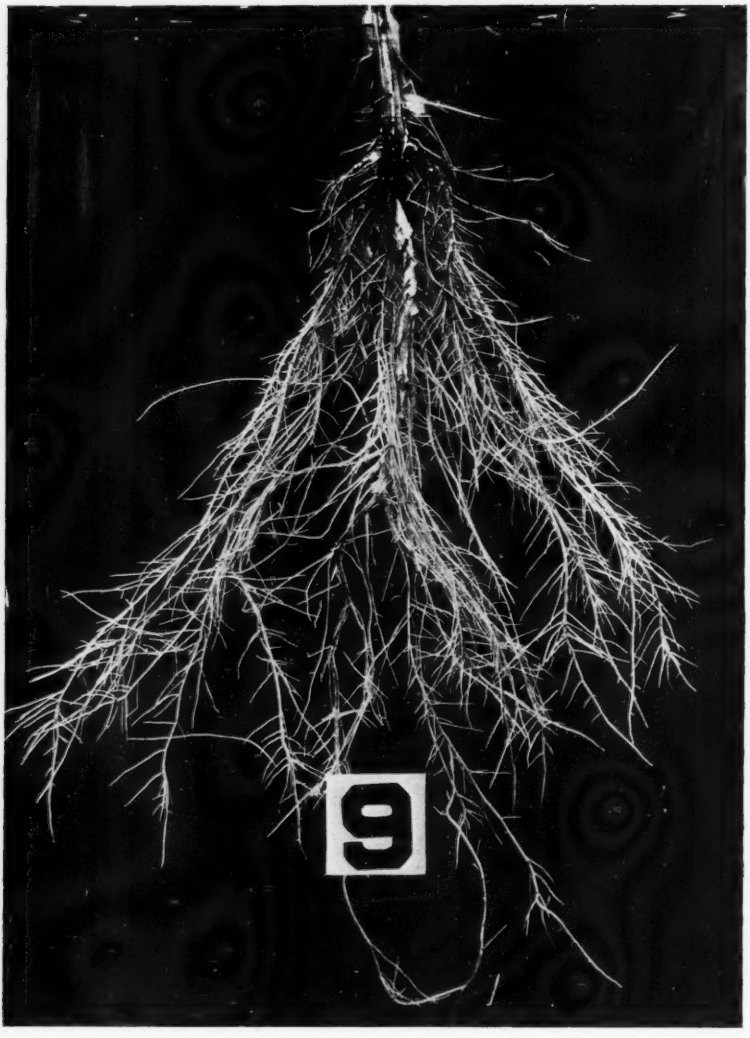
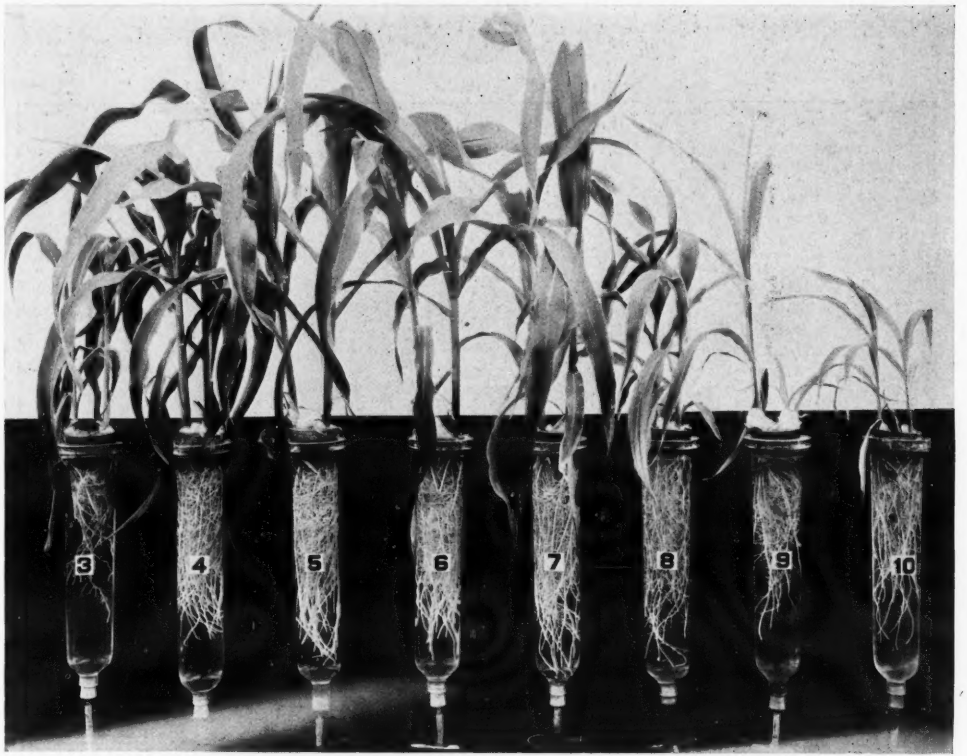


SOYBEAN ROOTS GROWN IN SOLUTION CULTURE WITH pH VALUE OF APPROXIMATELY 8

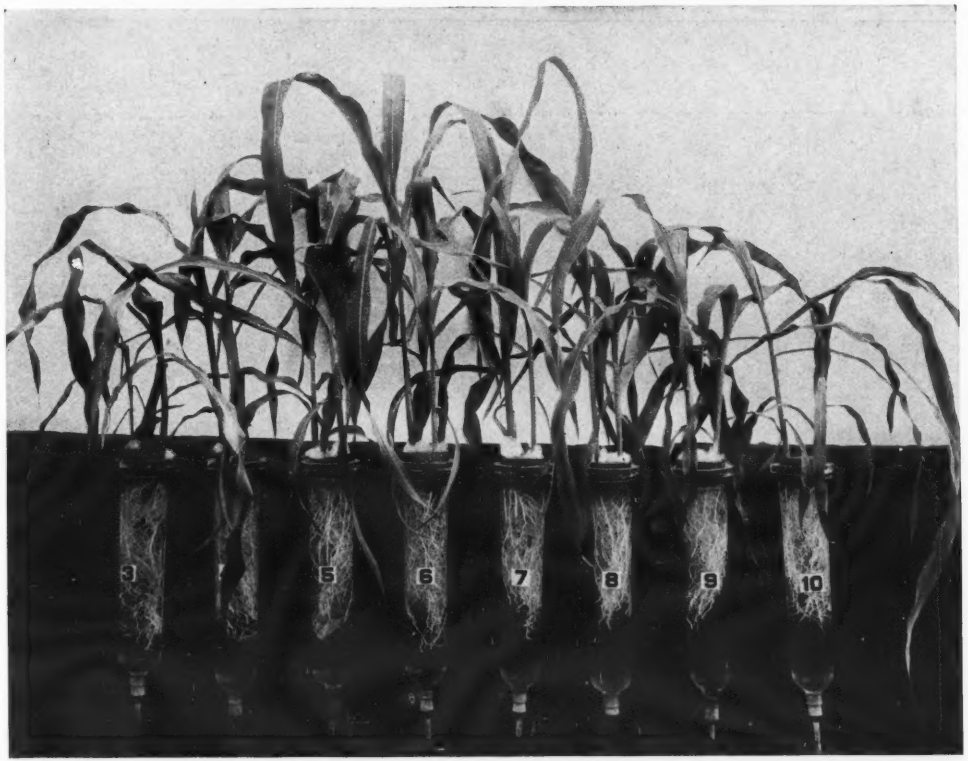


SOYBEAN ROOTS GROWN IN SOLUTION CULTURE WITH pH VALUE OF APPROXIMATELY 9

SOYBEAN ROOTS GROWN IN SOLUTION CULTURE WITH pH VALUE OF APPROXIMATELY 10

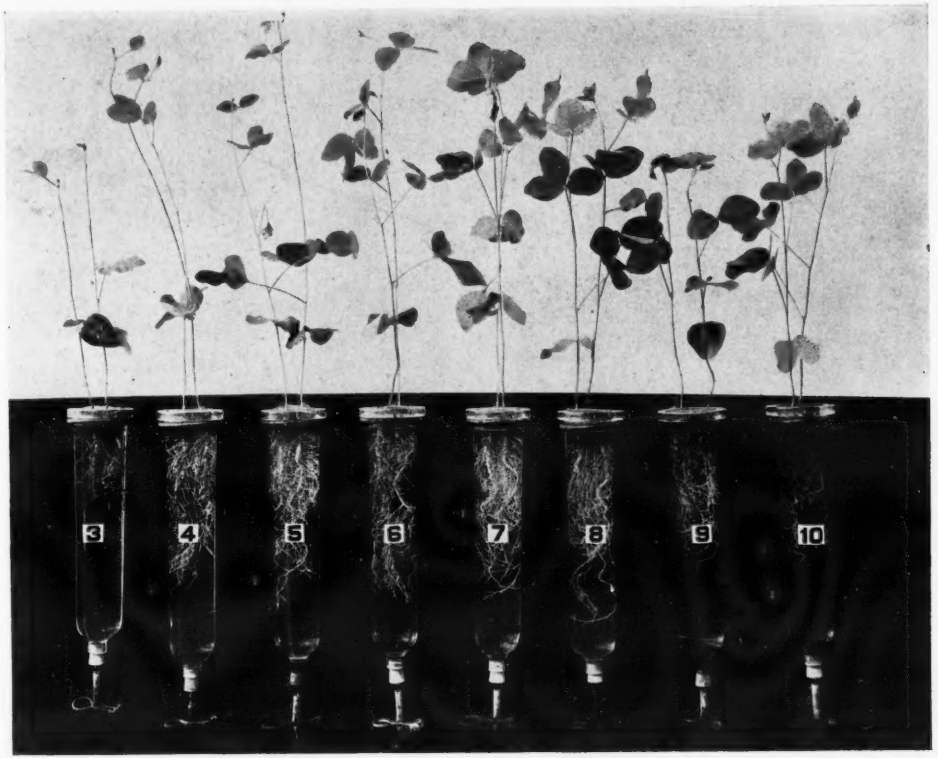


CORN GROWN IN SOLUTION CULTURES OF DIFFERENT REACTIONS. THE NUMBERS GIVE THE APPROXIMATE pH VALUES OF THE CULTURES



CORN GROWN IN SAND CULTURES OF DIFFERENT REACTIONS. THE NUMBERS GIVE APPROXIMATE pH VALUES OF THE CULTURES

300



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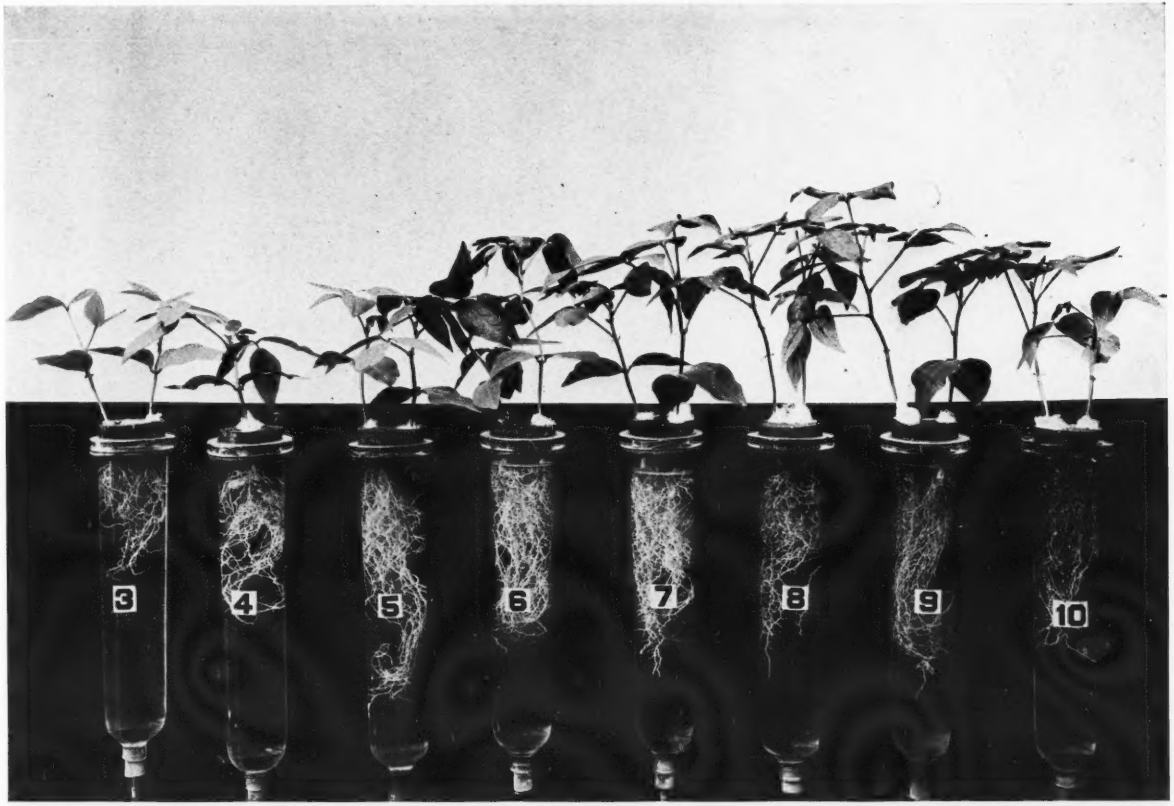
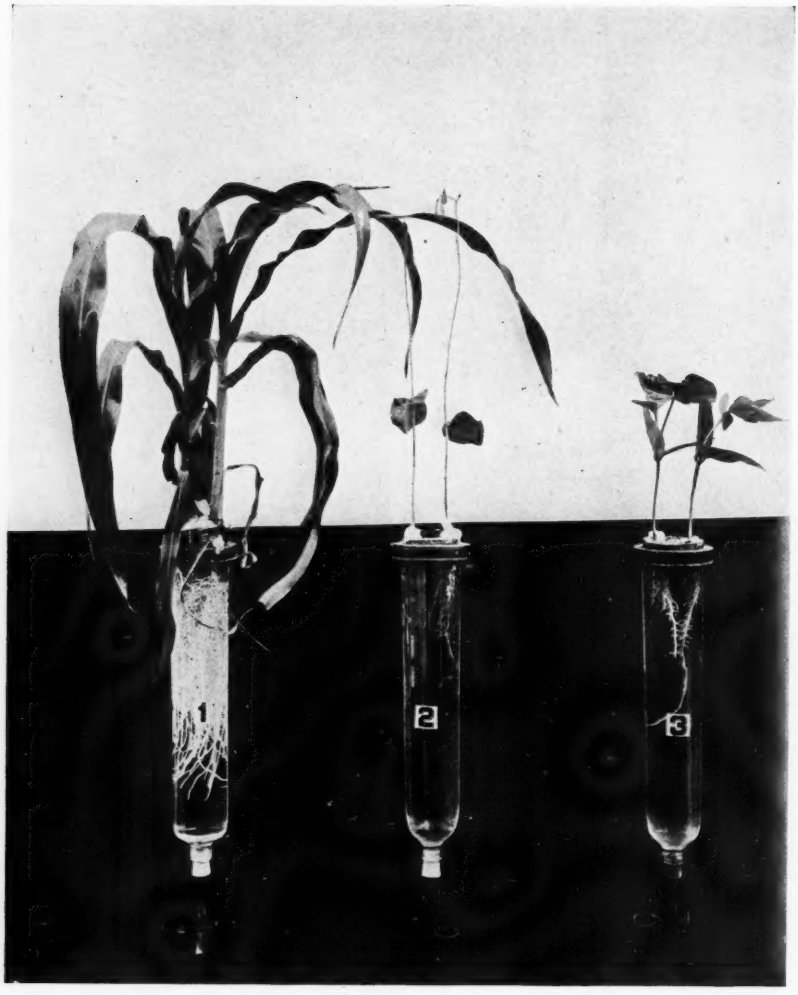


COWPEAS GROWN IN SOLUTION CULTURES OF DIFFERENT REACTIONS. THE NUMBERS GIVE APPROXIMATE pH VALUES OF THE CULTURES



CORN, SOYBEANS AND COWPEAS GROWN IN SOLUTION CULTURES OF APPROXIMATELY pH 4.0 IN EACH CASE

EFFECT OF TREE PRODUCTS ON BACTERIOLOGICAL ACTIVITIES IN SOIL: I. AMMONIFICATION AND NITRIFICATION

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INTRODUCTION

A large percentage of the soil in northern Idaho is a rather fine silt loam known technically as Helmer silt loam. This area is practically all in timber, but as the saw makes its inroads on the vast expanse of forest the acreage of land for agricultural purposes rapidly increases. This type of soil, apparently not markedly deficient in the essentials for plant growth, offers considerable difficulties when turned to agricultural use. The timber is usually removed *en masse* and the debris burned, thus the soil is cleared in the shortest possible time. It is then seeded to a suitable crop, such as clover, oats, wheat, or grass. Actual crop yields over a period of years are difficult to obtain, but it is the current opinion of those familiar with farming this area that the first crop yield on this type of soil is as good as could be expected considering the poor physical condition of the soil as the result of removing the timber. But each successive crop, in general, is poorer than the first even though the physical condition greatly improves with each crop. After three or four years better crops are obtained.

The fair yield obtained on the soil the first year after the removal of the timber is attributed to the small amount of readily available plant-food left in the soil. This readily available plant-food is removed in the first crop, and later crops are dependent upon the less available plant-food. The lack of productiveness for the next five to seven years is thought to be due to substances left in the soil by the timber which are deleterious to plant growth. The growing forest is thought to impart substances to the soil which are detrimental, and the accumulation of these products from year to year eventually results in a concentration which reacts upon the crop. These soils are known to the farmer and homesteader as "turpentine soils." The presence of turpentine has never been demonstrated, but it is thought that substances detrimental to plant growth exist in the soil as forest by-products, and that five to seven years of cropping is necessary to remove them.

REVIEW OF LITERATURE

A relatively small amount of information is available as to the effect of tree products on bacteriological activities in soil, and that much is more or less contradictory and does not

apply to Idaho conditions. The European forests which have been investigated relative to soil conditions are unlike our northwestern forests in that they contain variable amounts of humus material, somewhat peat-like, and have a very poor physical texture, while our forests are relatively free from peat and excessive amounts of organic matter, and have a good physical texture.

Baumann (1) states that it is not common to find nitrate in forest soils.

Boussingault (2) made a study of nitrate accumulation in forest soils and reported a very slight trace or none at all. His methods were later criticised by Weis (16).

Breal (3) tested the soils in beech forests at altitudes varying from 300 to 700 meters, and found only very slight traces of nitrate. He was of the opinion that nitrifying organisms formed only small amounts of nitrate, due to soil acidity, and the small amount formed was quickly removed by the luxuriant vegetation, which explained the absence of nitrate in the tests.

Ebermeyer (4) states that nitrifying organisms are not present in forest soils, due to their varying soil temperature and low moisture content.

Vogel von Falckenstein (5) found that light forest soils poor in lime produced but little nitrate, but that the amount formed was sufficient for the forest's demands. Forest soils not lacking in lime contained a larger amount of nitrate. Non-acid soils containing litter from pine and beech forests were able to gain in nitrate.

Grandeau (7) in a discussion based upon investigations by E. Henry, states that forest soils increase in nitrogen, but nitrification does not occur.

Hesselman (8), in a very extensive discourse on the forests of Denmark, states that the soils do not nitrify, and contain relatively no nitrogen as nitrate. The complete absence of nitrifying organisms was noted in many instances. The forests described by Hesselman contain a large amount of moss-like material over the soil, similar in some respects to our shallow peat. These conditions do not exist in the Idaho forests.

Koch (9) reported on studies of humus from pine and beech forest soils, and of the volatile oils and other products of conifers with reference to seed germination, the growth of plants, and the life and activity of yeasts and bacteria. Turpentine and carvene retarded the germination of corn, but had no marked effect after germination. Oil from the needles of silver pine was toxic. Ground pine needles added to the soil inhibited plant growth. Turpentine, resin, cedar oil, and various other products, exerted marked toxic action on nitrate formation from ammonium sulfate. These products also inhibited the development of yeasts in suitable media. The oxidation of ammonium sulfate was marked in beech humus, but small or negative in pine humus. He suggests that these substances may act as energy for the denitrifying group and thus prevent nitrate accumulation. He concluded that the majority of the substances tested were toxic to higher plants, yeasts, and bacteria, and that pine humus had a general unfavorable effect on plant growth. In later work (10) he tested the effect of colophony, resin, and tannin on biological transformations in soil. Pine resin acted as a source of energy for the nitrate reducing organisms, which he gave as an explanation of the low nitrate content of resinous soils. Resin was thought to hinder nitrification. Tannin was assimilated by molds and fungi, which caused a temporary loss of nitrate.

Migula (11) prepared decoctions of leaves and pine straw containing 0.5 per cent peptone and 0.5 per cent sugar. These were inoculated with soil and produced vigorous bacterial growth and acid formation. He attributes the decomposition of these substances in forest soil to the action of fungi. Surface layers of the soil contained few bacteria because of humus acids produced in the decomposition of the forest materials by fungi.

Moriya (12) analyzed the fallen leaves from various trees in forests, chiefly sugi (*Cryptomeria japonica*), akamatsu (*Pinus densiflora*), and kuramatsu (*Pinus thunbergii*). Some of the leaves contained as high as 0.9 per cent nitrogen. The soils bearing these forests were found to contain a high amount of nitrogen.

Muller and Weis (13) found that nitrification in beech humus was negligible unless treated with calcium carbonate, in which case it proceeded normally.

Parrozzoni (14) studied soils from forests of beech, cork oak, chestnut, and broom. Nitrate was found in all the soils except broom, while the highest amount was found in cork oak. Five soils were rich in nitrate and five were not.

Räuber (15) found ten to twenty times as many bacteria in calcareous forest soils as in sandy forest soils. There were very few organisms in the surface layer of soil due to the humic acids present from the decomposition of forest litter.

Weis (16) found nitrate in the soils of Denmark in amounts varying with the season. He states that nitrification in these soils is dependent upon the addition of lime. Soils under forest were found to contain as high as 65 mgm. of nitrate nitrogen per kilogram.

Zemplen and Roth (17) state that nitrogen is fixed by a hair-like growth on the forest trees, and the soil nitrogen supply is not only maintained but increased due to this fixation.

PURPOSE OF INVESTIGATION

Our aim in this investigation was to determine the presence or absence of substances in the various coniferous and deciduous trees, common to the Idaho forests, which are deleterious to bacteriological activities in soil. If such substances are present in the various trees, or arise from the decomposition of products from the trees, their accumulation from year to year would ultimately lead to an aggravated toxic condition in the soil. Fernow (6) states that an acre of forest produces 8,000 to 10,000 pounds of dry matter per year, of which half or more is wood above ground and 450 pounds is roots, leaving approximately 3,000 pounds of leaves per year. A forest 100 years of age would then have produced a total of 300,000 pounds of leaves, which is 15 pounds per 100 pounds of soil (acre 6 inches), or 15 per cent. It is, of course, impossible to estimate with accuracy the weight of needles which would fall on the soil from a given forest, and it would in all probability not be 15 per cent of the acre 6 inches of soil, but many leaves do fall, and to these are added the bark, twigs, cones, and other products, so that the actual weight of material entering the soil is no small amount. The undecomposed material representing the floor under the average forest may at times be several inches in thickness. The rainfall in this section of the northwest is never sufficient to wash away any appreciable amount of material comprising the forest floor, and leaching of products accumulating in the soil does not take place as in the mid-west and corn belt areas. In case these substances are detrimental to bacteriological activities in the soil, or their decomposition results in a toxic material, the soil would have ample opportunity to be heavily impregnated. This work was undertaken to determine if toxicity would develop as a result of applications of various forest materials to the soil, and the extent of this action. No attempt has been made to isolate material proving toxic. We have used various forest materials in amounts varying from 1 to 3 per cent. This is well below the amount we could expect to find in the soil naturally, based on the statements of Fernow.

EXPERIMENTAL DATA

Woods and tree products

The Idaho forests contain western red cedar (*Thuya plicata*), western white pine (*Pinus monticola*), western yellow pine (*Pinus ponderoso*), Douglas or red fir (*Pseudosuga taxifolio*), lowland or white fir (*Abies grandis*) and western larch (*Larix occidentalis*). White ash (*Frexinus americana*), and hard maple (*Acer saccharum*) were included in this work as representatives of the hard woods, but they are not common to the Idaho forests. Sawdust from kiln-dried boards made from all these timbers after the bark had been removed, was obtained, passed through a 10-mesh sieve, and then stored in paper bags in a dry room. These woods were used in varying amounts, as will be described later. After the work had progressed for some time, additional tree products were collected. These consisted of leaves, needles, cones, and bark from the above trees, and "forest floor" and fern brakes (*Pteris aquilina* L.). The needles were carefully trimmed from the twigs, air-dried, finely ground, and used in the same amounts as the other materials. The fern brakes were likewise treated. They were collected because it is well known that they are abundant on cut-over land, and are also quite poisonous if eaten in any considerable quantity. They can have no economic importance in this work but are included as a matter of interest. The "forest floor" consisted of an indiscriminate collection of the material underlying the forest, which forms a soil covering varying in depth with the density of the forest. It was composed chiefly of needles, leaves, bark, small twigs, and scattered cones, which were somewhat dry and brittle from exposure to the elements. The bark was collected from logs going into the mill, therefore was not kiln-dried as was the sawdust. All materials, after drying, were finely ground and stored in paper bags in a dry room. In a few experiments needles and leaves were used before they were completely dry. These will be designated in the data to follow.

Soils

Samples of Helmer silt loam the supposedly toxic timber soil were collected near the center of a cut-over area. The soil is a grayish or brownish yellow to pale yellow silt loam to a depth of 6 to 10 inches, underlaid to a depth of 24 to 30 inches by a non-calcareous pale yellow to yellowish-gray, powdery silt loam. This passes into a yellowish-gray to drab compact silt loam to silty clay loam, which usually extends to a depth of 36 to 50 inches. The surface soil contains a relatively small amount of decomposed organic matter and a relatively large amount of small roots, bits of wood, and other material from the forest. This material was largely screened out in the preparation of the sample for laboratory experiments. This particular field had been cleared for two years but was cropped only the year previous when it was seeded to oats. The yield was fair. The physical condition was unusually good con-

sidering the fact that the timber had been recently removed. Acidity as determined by the Truog method showed the soil to be medium acid.

Timber, garden, and field soils were chosen to determine the effects of the various forest materials on bacteriological activities.

This timber soil is unlike Helmer silt loam in origin and topography. It has a covering of loessal material underlaid with calcareous material of lake-bed origin at a depth varying from 10 inches to 2 feet. It represents a large acreage of soil in northern Idaho, and like the Helmer silt loam is a forest soil which produces very poorly. This type was collected from four different localities, each sample being used separately in experiments. The results obtained with this type of soil are the work of the previous bacteriologist in charge.[1] The soil was found to be medium acid by the Truog test.

The garden soil was a very rich one from the experiment station farm. It had been heavily manured two years previous, and some of the manure was still undecomposed in the soil. This manure was carefully avoided in the selection of the sample and that which was included was largely screened out in the later preparation of the sample. This soil was slightly acid by the Truog test.

The field soil was taken from a corn field at the experiment station farm in August 1920. This type represents a large acreage in northern Idaho—a fertile silt loam of light color and great depth—which never fails to produce well. It was found to be slightly acid by the Truog test.

All soil samples were carefully screened and stored in galvanized iron cans until used. The moisture content at the time of collection was approximately 15 per cent in all cases. This moisture content was maintained in the stored sample. They were stored in a building entirely separate from the laboratory and the temperature was approximately that found in the field.

METHODS

Ammonification

One-hundred-gram samples of the soil were weighed into wide-mouth bottles of 500 cc. capacity. One gram of dried blood was added to each, the contents thoroughly stirred, and the moisture brought to 25 per cent. An additional 2 gm. of water was added for each gram of blood and each gram of wood or other tree product under test. The bottles were plugged with cotton and incubated at 28°C. for 24 hours, then thoroughly stirred to insure equal distribution of moisture and other materials. They were then incubated for an additional 6 days, at the end of which period they were analyzed for ammonia content. This was determined by transferring the samples to copper flasks of 800-cc. capacity, adding approximately 5 gm. of heavy magnesium oxide and a small piece of paraffin to prevent foaming, and distilling the contents into

[1] T. L. Hills, former Assistant Bacteriologist, Idaho Agricultural Experiment Station.

standard sulfuric acid. The receiving flasks were transferred to a hot plate and boiled to approximately 100 cc. The excess acid was then titrated, using methyl red as indicator.

Nitrification

The samples for nitrification were prepared in the same manner as those for ammonification except that the moisture content was made 20 per cent. In some of the experiments ammonium sulfate, casein, and gelatin were used as the nitrifiable substance. In those cases the nitrogenous material was added from sterile standardized solution by volume at the same time the water was added to bring the moisture to 20 per cent. The samples were incubated at 28°C. for 4 weeks. Each week the moisture lost by evaporation was restored. At the end of the incubation period the samples were removed from the incubator, sufficient water added to bring the total water content to 400 cc., and the samples shaken at 15-minute intervals for 2 hours. Calcium oxide was then added as a flocculating agent, and the contents filtered through folded filters. Aliquots of the filtrate were evaporated to dryness in porcelain evaporating dishes and nitrate determined by the phenolsulfonic acid method.

In a few preliminary experiments sterile glass tumblers with petri dish covers were used as containers for the individual samples. The superiority of the wide-mouth bottle as a time and labor saver was soon recognized. The bottle plugged with cotton also affords better and more uniform aeration.

Denitrification

The samples for denitrification were prepared the same as those for ammonification. Definite amounts of standard sodium nitrate in solution were used instead of ammonium sulfate or dried blood.

RESULTS

Ammonification and nitrification, Helmer silt loam

Preliminary tests were made with the sample of Helmer silt loam to determine its ammonifying and nitrifying power. Thirty milligrams of nitrogen in the form of casein and blood was used to determine the ammonifying power, both with and without calcium carbonate. For determining the nitrifying power, 30 mgm. of nitrogen in the form of gelatin, casein, ammonium sulfate, and 1 per cent blood were used, each with and without calcium carbonate. The results of these tests are set forth in tables 1 and 2.

Table 1 shows that the supposedly toxic timber soil, Helmer silt loam, is not deficient in its power to convert organic nitrogenous material to ammonia. In the samples containing casein 95.4 per cent of the nitrogen was converted to ammonia. The efficiency of the soil to convert nitrogen in the form of casein or blood to ammonia is not increased by the addition of lime.

A study of the data in table 2 reveals the fact that the nitrifying power of this soil is very poor indeed. Whether it is the result of forest growth, or other cause, the fact remains that its power to convert organic nitrogenous material or ammonium sulfate to nitrate is practically nil. This condition in the soil is corrected by the application of calcium carbonate. The ability of the soil to form nitrate from casein, blood, and gelatin is not due to lack of ammonification of these substances, as is seen from the data in table 1. The acidity of the soil is no doubt a large factor in the low nitrate formation; samples of Helmer silt loam from many different localities with varying degrees of acidity have been used in these tests and results in accord with those presented above have been obtained.

TABLE 1

Ammonification in Helmer silt loam, timber soil

SAMPLE NUMBER	TREATMENT PER 100 GM. OF SOIL	NITROGEN AS AMMONIA
		mgm.
1– 4	None	1.40
5– 8	1 per cent $CaCO_3$	1.32
9–12	30 mgm. nitrogen (in casein)	28.60
13–16	30 mgm. nitrogen (in casein) and 1 per cent $CaCO_3$	27.70
17–20	1 per cent blood	35.35
21–24	1 per cent blood and 1 per cent $CaCO_3$	33.55

TABLE 2

Nitrification of various substances in Helmer silt loam, timber soil

SAMPLE NUMBER	TREATMENT PER 100 GM. OF SOIL	NITROGEN AS NITRATES	
		No $CaCO_3$	$CaCO_3$ 2 per cent
		mgm.	*mgm.*
1– 6	None	3.63	4.90
7–12	1 per cent blood	7.27	42.10
13–18	30 mgm. nitrogen (in gelatin)	7.11	23.88
19–24	30 mgm. nitrogen (in casein)	8.00	28.57
25–30	30 mgm. nitrogen (in ammonium sulfate)	4.77	36.36

Effect of sawdust on ammonia accumulation from blood in timber soil

The four samples of timber soil previously described were used to determine the effect of sawdust, with and without calcium carbonate, on ammonia accumulation from blood. Since the results secured with each sample were similar, only the average of the four samples is given in figure 1. The sawdust was applied at the rate of 1.5 gm. to 100 gm. of soil to which the blood and calcium carbonate were applied at the rate of 1 gm. per 100 gm. of soil.

It is seen from the data in figure 1 that sawdusts from the various woods reduced ammonia accumulation. Cedar was decidedly most toxic, while maple was next, with the other woods following with more or less toxicity.

Cedar reduced ammonia accumulation 78.9 per cent, while white pine, the least detrimental of the group, reduced it 13.1 per cent. Calcium carbonate tends to decrease the toxicity, but in no case is the ammonia accumulation equivalent to that in the control without the calcium carbonate. Ammonification in this soil is very good, better, in fact, than in the productive field soil used in later experiments.

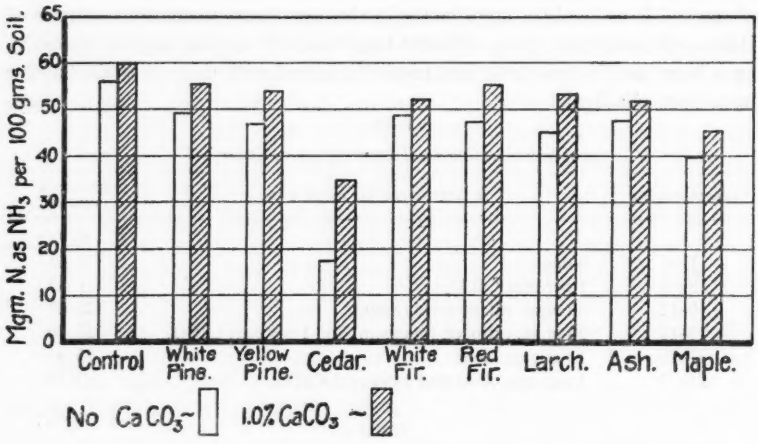

FIG. 1. EFFECT OF SAWDUST ON AMMONIA ACCUMULATION FROM BLOOD IN TIMBER SOIL

Effect of woods and tree products on ammonia accumulation from blood in field soil

The foregoing ammonification experiments to test the effect of woods were repeated, using the productive field soil previously described. The quantities of the various tree products used were 1.0 and 1.5 per cent of the weight of the soil sample; the quantity of blood used was 1.0 per cent. Results are given in figure 2 which show that all treatments reduced ammonia accumulation except the 1 per cent applications of white pine, yellow pine, and white fir sawdusts and larch needles. Of the sawdusts, cedar exerted the greatest retarding influence followed by red fir, maple, and larch. This agrees closely with the results obtained with the timber soil, in which case cedar was followed by maple and larch, with the others grouped near fourth place. Among the tree products other than sawdust, cedar needles were the most detrimental, followed by yellow pine, white pine, and white fir needles. Larch needles, and white and yellow pine sawdusts, show but little retarding influence.

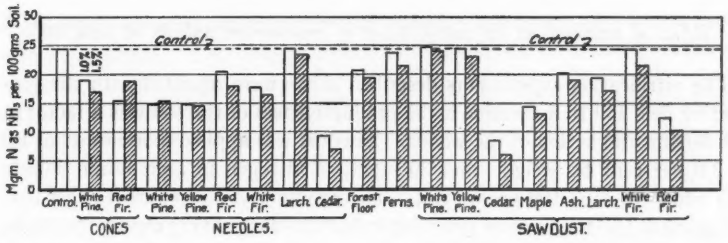

FIG. 2. EFFECT OF TREE PRODUCTS ON AMMONIA ACCUMULATION FROM BLOOD IN FIELD SOIL

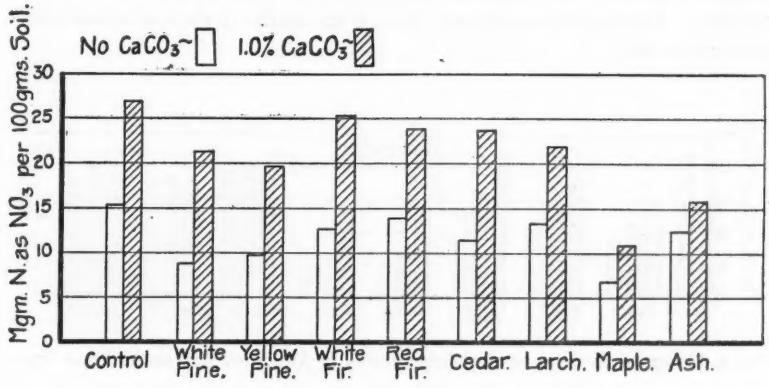

FIG. 3. EFFECT OF SAWDUST ON NITRIFICATION OF AMMONIUM SULFATE IN TIMBER SOIL

Effect of sawdust on the nitrification of ammonium sulfate in timber soil

One-hundred-gram samples of timber soil were treated with 1.5 per cent sawdust from the various woods and 30 mgm. nitrogen in the form of ammonium sulfate, both with and without 1.0 per cent calcium carbonate. Four samples of soil were used. The average results are given in figure 3 which show that all treatments inhibited nitrate formation from ammonium sulfate. In the absence of calcium carbonate maple caused the greatest retardation, with white pine, yellow pine, and cedar following in order. This is not the order of toxicity found in the ammonification experiments, white pine having the least influence in that case. In the presence of calcium carbonate the order of toxicity is somewhat changed, maple having the greatest retarding influence, followed by ash, yellow pine, and white pine. There is a marked response to the application of calcium carbonate. The detrimental action of the various woods is in no sense overcome, however, since the response in the control is equally as great as in the others. This is probably due to the fact that the calcium carbonate corrects natural conditions in the soil unfavorable to the nitrifying organisms.

Effect of sawdust on the nitrification of ammonium sulfate in field soil

The nitrification experiments described above were repeated with the productive field soil to determine if the productiveness of the soil was a factor in the detrimental action of the woods. Sawdust was applied in amounts of 1.0 to 3.0 per cent by weight, and 30 mgm. of nitrogen in ammonium sulfate was added to each 100 gm. of soil. The results given in figure 4 show that ash sawdust had the greatest retarding influence followed very closely by maple, then cedar, white and yellow pine. Three per cent of ash and maple sawdust stopped practically all nitrate formation, and 1 per cent reduced it by nearly one-half. The results secured with this soil are similar to those obtained with the timber soil.

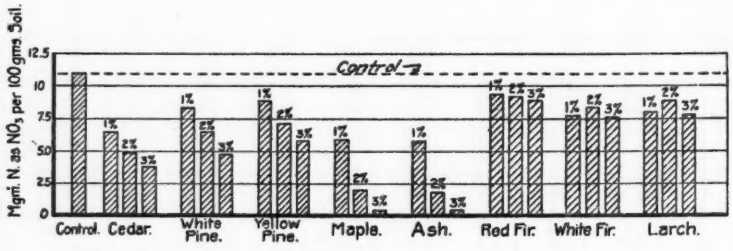

FIG. 4. EFFECT OF SAWDUST ON THE NITRIFICATION OF AMMONIUM SULFATE IN FIELD SOIL

Effect of sawdust on the nitrification of blood in field soil

Sawdust was applied to the field soil and garden soil previously described at rates of 1 to 2.5 gm. per 100 gm. of soil. All samples received 1 per cent of dried blood. The results obtained with the field soil are set forth in table 3 and are represented diagrammatically in figure 5, while those with the garden soil appear in table 4 and figure 6.

It will appear from a study of tables 3 and 4 that there is a discrepancy between amounts of wood 1.5 and 2.0 per cent, and that the 2.0 per cent has nitrified to a further degree than the 1.5 per cent. A more careful study will show this is not the case. The number of samples of soil to handle in connection with the work was so great it became necessary to run concentrations 1.0 and 1.5 per cent at one time, and 2.0 and 2.5 per cent at a later date. The controls run at different times did not always form the same amount of nitrate, although conditions were controlled as well as could be. It will be noted in table 3 that the control for 1 and 1.5 per cent sawdust was 31.7 mgm. nitrogen, while the control for 2 and 2.5 per cent,which was run at a later date, was 36.3 mgm. nitrogen. The double line between concentrations 1.0 and 1.5 per cent on the one side, and 2.0 and 2.5 per cent on the other, indicates that they were run at different times. The data in these tables are converted to percentage

efficiency, considering the control in each case as 100 per cent, and are set forth in charts 5 and 6. This change makes comparable the data accumulated at various times.

It is seen from the data in tables 3 and 4, and figures 5 and 6 that all the sawdusts tended to reduce nitrification of blood. In general this action increases with increasing amounts of sawdust, but not always. The experiments were repeated many times and irregularities were always noted. Cedar was the most toxic, followed by maple, larch, ash, and the pines. The order of toxicity was the same for both soils, even though the garden soil contained a large amount of organic matter and should have been better able to overcome the toxic material.

TABLE 3

Effect of sawdust on the nitrification of blood in field soil

SOURCE OF SAWDUST	NITROGEN AS NITRATE PER 100 GM. OF SOIL			
	1 per cent*	1.5 per cent*	2 per cent*	2.5 per cent*
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	31.7	31.7	36.3	36.3
White pine	29.1	28.0	31.9	30.9
Yellow pine	29.6	28.0	29.1	29.1
Cedar	16.3	12.3	10.4	8.4
Maple	26.9	23.7	24.5	21.0
Ash	27.6	30.1	31.7	30.5
Larch	27.8	26.9	32.7	30.5
White fir	29.2	31.2	35.5	35.1
Red fir	31.0	30.0	31.1	29.8

* The percentages refer to the amount of sawdust mixed with the soil and are based on the weight of the soil sample.

TABLE 4

Effect of sawdust on the nitrification of blood in garden soil

SOURCE OF SAWDUST	NITROGEN AS NITRATE PER 100 GM. OF SOIL			
	1 per cent*	1.5 per cent*	2 per cent*	2.5 per cent*
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	66.6	66.6	49.2	49.2
White pine	58.3	60.8	41.0	39.8
Yellow pine	63.6	63.6	39.0	40.3
Cedar	63.6	58.3	17.3	10.7
Maple	63.6	56.0	29.4	31.1
Ash	63.6	63.6	38.6	38.0
Larch	60.8	63.6	38.6	34.4
White fir	63.6	60.8	33.0	47.1
Red fir	63.6	63.6	41.1	45.0

* The percentages refer to the amount of sawdust mixed with the soil and are based on the weight of the soil sample.

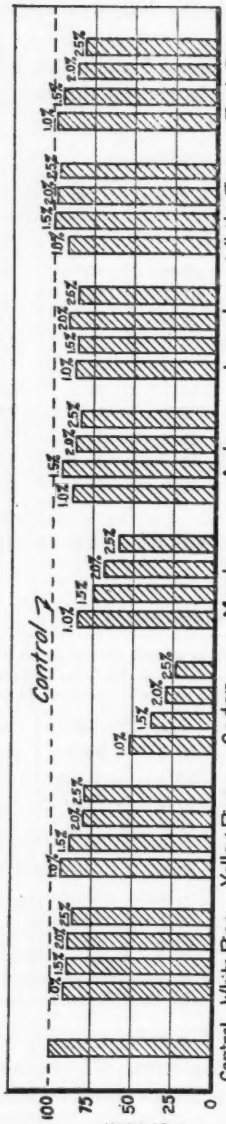

FIG. 5. EFFECT OF SAWDUST ON THE NITRIFICATION OF BLOOD IN FIELD SOIL

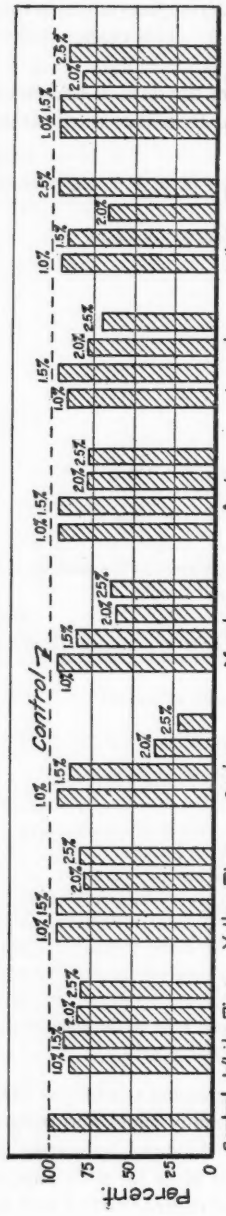

FIG. 6. EFFECT OF SAWDUST ON THE NITRIFICATION OF BLOOD IN GARDEN SOIL

It will be noted that the order of toxicity of the sawdusts is very similar in charts 4 and 5.

Effect of sawdust on the nitrification of blood in the presence of calcium carbonate in field and garden soils

The experiments to determine the effect of sawdust on the nitrification of blood with both field and garden soil were repeated with the addition of 1 gm. of calcium carbonate for each 100 gm. of soil. The results are given in table 5.

TABLE 5

Effect of sawdust on the nitrification of blood in the presence of 1.0 per cent calcium carbonate in field and garden soils

SOURCE OF SAWDUST	NITROGEN AS NITRATE PER 100 GM. OF SOIL			
	Field soil		Garden soil	
	1 per cent*	1.5 per cent*	1 per cent*	1.5 per cent*
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	75.8	75.8	123.9	123.9
White pine	72.7	70.3	123.9	118.6
Yellow pine	67.2	69.4	118.6	120.6
Cedar	64.0	56.9	120.6	107.7
Maple	58.7	52.8	107.7	96.5
Ash	64.3	61.4	118.6	112.0
Larch	70.8	64.3	116.6	113.8
White fir	68.1	67.2	86.8	86.8
Red fir	68.5	70.3	105.5	116.6

* The percentages refer to the amount of sawdust added to the soil and are based on the weight of the soil sample.

A great response to the application of calcium carbonate is noted in both soils. The toxic action of the various sawdusts is in no case overcome by the calcium carbonate but it was not nearly so marked as in its absence. The amount of nitrate formed was larger in the presence of each sawdust with lime than in the control without lime and sawdust. The order of toxicity was not the same with each soil. Maple, cedar, ash and white fir were, however, the most detrimental in each soil.

Effect of tree products on the nitrification of ammonium sulfate in field soil

One-hundred-gram samples of the field soil were treated with 30 mgm. of nitrogen in ammonium sulfate and the various forest products in amounts ranging from 1 to 3 per cent to determine the effect on nitrate formation. The results are given in figure 7.

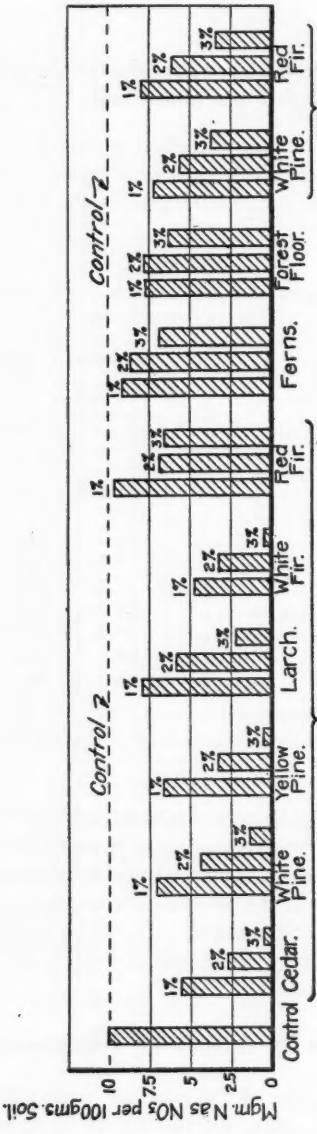

FIG. 7. EFFECT OF TREE PRODUCTS ON THE NITRIFICATION OF AMMONIUM SULFATE IN FIELD SOIL

The data in figure 7 show an inhibiting action by all the substances tested. White fir needles proved the most toxic, followed by cedar needles, and yellow and white pine needles. Ferns proved the least detrimental. It is interesting to note that white fir sawdust was the least toxic of the sawdusts towards nitrate formation, while the needles proved the most toxic. The addition of 3 per cent white fir, yellow pine, and cedar needles practically eliminated nitrate formation from ammonium sulfate.

Effect of tree products on the nitrification of blood in field and garden soils

The various forest products were applied to the soil in amounts ranging from 1 to 3 per cent to determine their effect on nitrification of blood. The

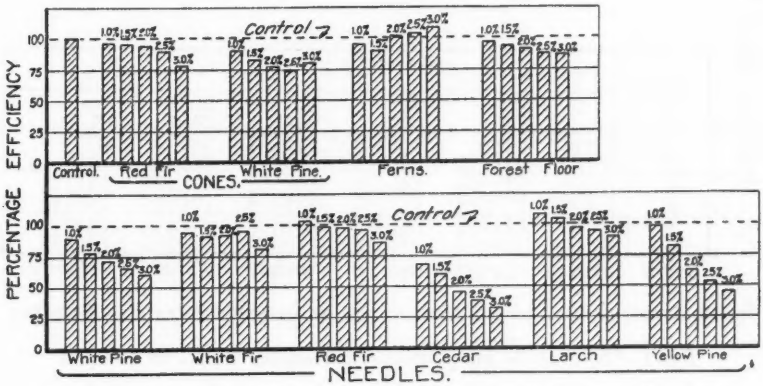

FIG. 8. EFFECT OF TREE PRODUCTS ON NITRIFICATION OF BLOOD IN FIELD SOIL

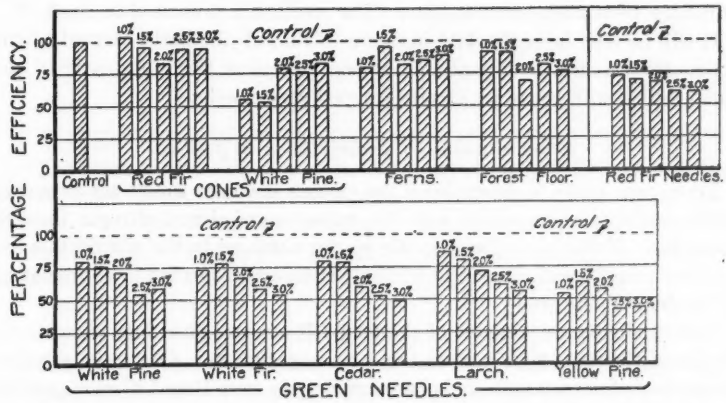

FIG. 9. EFFECT OF TREE PRODUCTS ON THE NITRIFICATION OF BLOOD IN GARDEN SOIL

results are given in figures 8 and 9, expressed in percentage efficiency considering the control as 100 per cent efficient.

It is seen from the data in figures 8 and 9 that the various products do not exert as marked a toxic action toward nitrification of blood as of ammonium sulfate, and also that the order of toxicity is not the same in the two soils. In the field soil cedar needles are quite toxic, followed by yellow and white pine needles. Red fir cones, ferns, larch needles, and red fir needles show little toxicity. In the garden soil the order of toxicity is yellow pine, white fir, red fir, white pine, and cedar needles. Red fir cones and ferns are very slightly toxic. Here again the white fir needles proved among the most toxic while the sawdust exerts but little toxicity in the same soil.

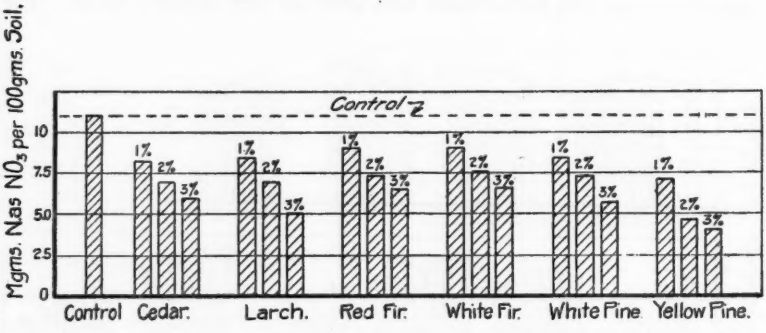

FIG. 10. EFFECT OF BARK ON THE NITRIFICATION OF AMMONIUM SULFATE IN FIELD SOIL

Effect of bark on the nitrification of ammonium sulfate in field soil

The finely ground bark, previously described, was applied to the soil in amounts ranging from 1 to 3 per cent to determine the action on nitrate formation from ammonium sulfate. The results are presented in chart 10.

It will be seen from the data in figure 10 that all the barks exerted a toxic action toward nitrate formation. Yellow pine was the most toxic of the barks tested, with the other species grouped very closely.

Effect of woods on denitrification in field soil

Tests were made to determine if the various sawdusts would act as energy for the denitrifying organisms and thus cause loss of nitrate nitrogen through reduction. If this were the case the results obtained in the nitrification experiments would not be due to a toxicity to those organisms but to a stimulation of the denitrifying group.

One-hundred-gram samples of the field soil were weighed into wide-mouth bottles of the same type used in the other experiments. One series received amounts of nitrogen as nitrate in amounts ranging from 10 to 120 mgm. per 100 gm. of soil. In a second series all samples received 30 mgm. nitrogen as nitrate

and 1.5 gm. of sawdust but a different kind of sawdust was used in each case. In the third series 1.5 gm. of cedar sawdust was applied to each sample, and the nitrogen varied from 10 to 120 mgm. The fourth series was the same as the third except yellow pine was used in the place of cedar. All samples were run in quadruplicate. The results are given in table 6.

TABLE 6

Effect of woods on denitrification in field soil

NITROGEN AS NITRATE PER 100 GM. OF SOIL	SAWDUST (1.5 PER CENT)	NITROGEN AS NITRATE RECOVERED FROM 100 GM. OF SOIL	LOSS OR GAIN IN NITRATE
mgm.		*mgm.*	*mgm.*
10	None	16.26	6.26
20	None	23.53	3.53
30	None	33.33	3.33
60	None	64.51	4.51
120	None	111.11	−8.89
30	None	33.70	3.70
30	Cedar	33.33	3.33
30	Maple	20.70	−9.30
30	Ash	26.50	−3.50
30	Larch	29.40	−0.60
30	White pine	29.70	−0.30
30	Yellow pine	28.20	−1.80
30	Red fir	32.26	2.26
30	White fir	31.22	1.22
10	Cedar	14.09	4.09
20	Cedar	25.64	5.64
30	Cedar	34.48	4.48
60	Cedar	60.60	0.60
120	Cedar	109.17	−10.83
10	Yellow pine	10.72	0.72
20	Yellow pine	18.18	−1.82
30	Yellow pine	32.26	2.26
60	Yellow pine	62.50	2.50
120	Yellow pine	111.11	−8.89

It will be seen from the data in table 6 that maple, and possibly ash, are the only sawdusts which caused loss of nitrate. This experiment indicates that the toxicity obtained in the ammonification and nitrification experiments is not due to the action of denitrifying organisms, with the exception of maple, and possibly ash, but to a toxicity, or inhibition of the specific groups.

SUMMARY

Sawdusts from cedar, maple, ash, red fir, white fir, larch, and white and yellow pine, were collected from kiln-dried boards, carefully screened and used in amounts varying from 1 to 3 per cent to determine the effect on ammonia

accumulation from dried blood, nitrification of ammonium sulfate and dried blood and nitrate reduction in forest soils. Other tree products, such as leaves, needles, and cones from the above named trees, "forest floor," and ferns were similarly tested. Three types of soil were used in the tests, namely, a timber soil of low fertility, a field soil of average fertility, and a garden soil of high fertility.

All the substances under test reduced ammonia accumulation. In the timber soil cedar caused the greatest reduction, followed by maple. Cedar reduced ammonia accumulation 78.9 per cent when applied at the rate of 1.5 per cent by weight, while white pine, the least toxic of the group, reduced it but 13.1 per cent. The results with the field soil were very similar to those obtained with the timber soil. Cedar, maple, and larch were the most toxic. Among the products other than sawdust cedar needles exerted the greatest inhibitory action, followed by yellow pine, white pine, and white fir needles. Larch needles reduced ammonia accumulation only slightly.

All the substances tested proved inhibitory to nitrate formation from blood and ammonium sulfate. This action is not overcome by application of calcium carbonate. Ash and maple sawdust in 3 per cent applications practically stopped nitrification of ammonium sulfate in the field soil. Cedar, maple, larch, ash, and red fir sawdusts caused the greatest reductions in nitrification of blood and ammonium sulfate. Among the products other than sawdust, 3 per cent white fir, yellow pine, and cedar needles practically eliminated nitrate formation from ammonium sulfate. White fir sawdust was but slightly detrimental, while the needles were extremely so. Bark from the trees tested reduced nitrate formation from ammonium sulfate. The "forest floor," which consisted of the indiscriminate collection of the material underlying the forest and covering the soil, reduced nitrate formation from ammonium sulfate and blood. Ferns, which are abundant on cut-over lands, proved to have but slight effect and would seem, therefore, to have no economic importance.

The results of the denitrification experiments indicate that none of the substances tested, with the exception of maple and possibly ash, serve as energy for the denitrifying organisms.

The results of the entire work indicate that the low fertility, and apparent toxic condition of the Helmer silt loam, is in a large part due to the timber residue. This material collects continuously throughout the growing period of the forest, and due to its slow rate of decomposition has a direct effect upon the beneficial biological processes in the soil. The quantities of tree products used in the treatments were, in all probability, below those actually occurring in the forest soil under timber. The accumulation of the forest materials from year to year leads to a concentration which is detrimental to ammonification and nitrification. Maple and ash are not common in the Idaho forests, but the other forest materials which showed marked toxic action are abundant. This is particularly true of cedar and the pines. It is com-

monly thought that a period of three to five years is required for this timber residue to completely decompose and its detrimental action to disappear. The use of sawdust bedding is to be discouraged in case the sawdust is to be spread with the manure on the field.

CONCLUSIONS.

The various tree products tested proved inhibitory to ammonia and nitrate accumulation in three different types of soil.

Calcium carbonate does not overcome the inhibiting action of the various materials tested, but causes a general increase in nitrate formation.

Cedar sawdust was the most inhibitory of the sawdusts to ammonia accumulation from blood. The reduction in the timber soil was 78.9 per cent, while white pine, the least inhibitory of the sawdusts, caused a reduction of 13.1 per cent.

Cedar, maple, larch, ash, and red fir sawdusts had the greatest inhibiting action upon nitrate formation from ammonium sulfate and blood.

Among the materials other than sawdust, white fir, yellow pine, and cedar needles caused the greatest reductions in the nitrification of ammonium sulfate.

The indiscriminate collection of material covering the soil under the forest, designated as "forest floor," reduced ammonia and nitrate accumulation in soil.

Ferns, common on cut-over soils, proved but slightly inhibitory and therefore of no economic importance.

Denitrification experiments indicated that the reduction of nitrate formation is not due to the denitrifying group, but to actual inhibition of the nitrifying organisms. Maple, and possibly ash, are exceptions, but are not common in the Idaho forests.

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